



Genetic analysis of a four generation Indian family with Usher syndrome: a novel insertion mutation in *MYO7A*

Arun Kumar,¹ Mohan Babu,¹ William J. Kimberling,² Conjeevaram P. Venkatesh³

¹Department of Molecular Reproduction, Development, and Genetics, Indian Institute of Science, Bangalore, India; ²Center for the Study and Treatment of Usher Syndrome, Boys Town National Research Hospital, Omaha, NE; ³Minto Ophthalmic Hospital, Bangalore, India

Purpose: Usher syndrome (USH) is a rare autosomal recessive disorder characterized by deafness and retinitis pigmentosa. The purpose of this study was to determine the genetic cause of USH in a four generation Indian family.

Methods: Peripheral blood samples were collected from individuals for genomic DNA isolation. To determine the linkage of this family to known USH loci, microsatellite markers were selected from the candidate regions of known loci and used to genotype the family. Exon specific intronic primers for the *MYO7A* gene were used to amplify DNA samples from one affected individual from the family. PCR products were subsequently sequenced to detect mutation. PCR-SSCP analysis was used to determine if the mutation segregated with the disease in the family and was not present in 50 control individuals.

Results: All affected individuals had a classic USH type I (USH1) phenotype which included deafness, vestibular dysfunction and retinitis pigmentosa. Pedigree analysis suggested an autosomal recessive mode of inheritance of USH in the family. Haplotype analysis suggested linkage of this family to the USH1B locus on chromosome 11q. DNA sequence analysis of the entire coding region of the *MYO7A* gene showed a novel insertion mutation c.2663_2664insA in a homozygous state in all affected individuals, resulting in truncation of MYO7A protein.

Conclusions: This is the first study from India which reports a novel *MYO7A* insertion mutation in a four generation USH family. The mutation is predicted to produce a truncated MYO7A protein. With the novel mutation reported here, the total number of USH causing mutations in the *MYO7A* gene described to date reaches to 75.

Usher syndrome (USH) named after the British ophthalmologist Charles Usher [1] is the most common hereditary form of combined blindness and deafness [2]. It is a rare disorder with an incidence of 3.5/100,000 in Scandinavia [3] to 4.4/100,000 in the USA [4]. It shows an autosomal recessive mode of inheritance. According to clinical symptoms, USH is classified into three types: USH type I, USH type II and USH type III. USH type I is the most severe form and is characterized by severe to profound congenital sensorineuronal deafness, constant vestibular dysfunction (balance deficiency) and prepubertal onset of retinitis pigmentosa (RP). USH type II has a congenital mild to moderate hearing loss, normal vestibular responses, and RP during the second decade of life. USH type III has a progressive hearing loss, variable vestibular problems and variable RP [5].

USH type I is genetically heterogeneous with seven known loci: USH1A on chromosome 14q [6], USH1B on chromosome 11q [7], USH1C on chromosome 11p15.1 [8], USH1D on chromosome 10q21-22 [9], USH1E on chromosome 21q21 [10], USH1F on chromosome 10 [11] and USH1G on chromosome 17q24-25 [12]. USH1B is the most common subtype and accounts for about 70% of all type I cases [13]. Of seven loci for USH type I, genes for only five loci (USH1B, USH1C,

USH1D, USH1F, and USH1G) have been isolated so far. USH1B is known to be caused by mutations in an unconventional myosin, the motor protein myosin VIIA [14]. Atypical USH, in which the hearing loss is progressive, is also known to be caused by mutations in the myosin VIIA [15]. USH1C, USH1D, USH1F, and USH1G are caused by mutations in PDZ73 (harmonin), CDH23 (cadherin 23), PCDH15 (Procadherin), and SANS genes, respectively [16-19].

USH type II is genetically heterogeneous with three known loci: USH2A on chromosome 1q41 [20], USH2B on chromosome 3p22-24.2 [21], and USH2C on chromosome 5q14.3-21.3 [22]. The USH2A gene coding for extracellular protein usherin has been shown to be responsible for disease phenotype linked to the USH2A locus [23]. Weston et al. [24] have recently isolated the gene, VLGR1 (MASS1) for the USH2C locus. A single locus, USH3 has been mapped to chromosome 3q21-25 for USH type III [25]. Joensuu et al. [26] have identified the gene for this locus, which codes for a cell-cell adhesion protein called clarin-1.

Genetic analysis of USH has been carried out in patients from several countries [5-35]. However, there is no report on the genetic analysis of any Indian family with USH. We report here genetic analysis of a four generation Indian family with members suffering from USH for the first time. Haplotype analysis suggested mapping of this family to the USH1B locus. DNA sequence analysis identified a novel insertion mutation in the *MYO7A* gene in this family.

Correspondence to: Dr. Arun Kumar, Department of Molecular Reproduction, Development, and Genetics, Indian Institute of Science, Bangalore, 560 012, India; Phone: 91-80-2 293 2998; FAX: 91-80-2 360 0999; email: arunk00@hotmail.com

METHODS

Subjects: We have ascertained a four generation consanguineous USH family from Bangalore, India (Figure 1). Thirteen living family members, including five affected individuals, were recruited for the study. Each individual underwent a detailed clinical examination for Usher syndrome. No other abnormalities were noticed in affected individuals other than Usher syndrome symptoms and their development and intelligence appeared to be normal. Informed consent was obtained for research following the guidelines of the Indian Council of Medical Research, New Delhi.

Genotyping and mutation analysis: Peripheral blood samples were drawn from all 13 individuals in Vacutainer EDTA tubes (Beckton-Dickinson, Franklin Lakes, NJ). Genomic DNA samples were isolated from peripheral blood samples using a Wizard® genomic DNA extraction kit (Promega, Madison, WI). Because this family is consanguineous, homozygosity by descent was sought and used to assess evidence of linkage to a particular locus. Linkage of consanguineous families to a locus is based on the observation that if all affected individuals of a family had the same homozygous haplotype for a locus, the family was considered linked. In order to determine if this family is linked to one of the known loci for Usher syndrome type 1, two to seven microsatellite markers were selected from their candidate regions and used to genotype the family [5,12]. The markers selected from the seven known loci are shown in Table 1. Genotyping was performed as described in Kumar et al. [36].

For mutation analysis of the *MYO7A* gene, a set of 48 PCR primers, which cover the entire coding region of this gene along with intron/exon junctions, were used. Sequences and PCR conditions of these primers are provided in Table 2. Mutation in this gene was identified by sequencing the PCR products from one affected individual from the family on an ABIprism A310 automated sequencer (PE Biosystems, Foster City, CA). Prior to sequencing, PCR products were purified on Auprep® PCR Purification columns (Life Technology Pvt. Ltd., New Delhi, India). PCR-SSCP analysis was used to see if the mutation segregated with the disease in the family and was not present in 50 normal control individuals as described in Kumar et al. [36].

TABLE 1. MICROSATELLITE MARKERS FROM THE CANDIDATE REGIONS OF SEVEN KNOWN USH1 LOCI

Locus	Marker
USH1A	D14S78, D14S250, D14S292, D14S260
USH1B	D11S1902, D11S4179, D11S4186, D11S4079, D11S906, D11S911, D11S527
USH1C	D11S921, D11S902, D11S4160, D11S899
USH1D	D10S529, D10S195, D10S202, D10S573
USH1E	D21S1905, D21S1914, D21S269, D21S1913
USH1F	D10S193, D10S1791, D10S220, D10S1790
USH1G	D17S785, D17S1807

Two to seven microsatellite markers from the candidate regions of seven known USH1 loci were used to genotype the present USH family to find homozygosity by descent, which indicated the linkage of this family to a particular USH1 locus.

TABLE 2. PCR PRIMER SEQUENCES, ANNEALING TEMPERATURES AND AMPLICON SIZES OF 48 CODING EXONS OF THE MYO7A GENE

Exon	Primer sequence (5' to 3')	Annealing temperature (°C)	Amplicon size (bp)
2	F: ccagccaggctcaaggcttcca R: gcaggaattttccaagagaacacc	66	241
3	F: cagagggatagggctgcctgga R: catggcctccatctcctttgatca	66	296
4*	F: gtgtctggctgccagagagctga R: agctgcacagcggacaagctcag	58	426
5	F: agcccaagagctttctagagtcaga R: gcacagtggagctctaggtccta	66	316
6 & 7	F: ctgggctgagttccagttgggtg R: ggagcaatacgggcagcaatcag	63	512
8	F: atctccagggctagtctcagtgatg R: aaggctgagctcgcagtagccag	68	269
9	F: gggtagactgactcctcttgcaac R: ggcaactgcactgccctggccag	68	260
10	F: gtggcagcctagctccttaggac R: aacctcagagggacagaagtcatg	68	214
11	F: ggggcaggctggcaggtgagcag R: acttcccaagggtagggcgagcaa	68	212
12*	F: caagggctggagcgcacaccag R: tccatattgggaaaggaatccccatg	58	285
13	F: ggtggggctgacaacaccttac R: aagcaggaaggaagctgtgcccac	68	318
14	F: catggagagaggggtgggtcaca R: gagcaggggaagggcagggccagc	68	239
15	F: gagggcctgcccagagctggtgaga R: gcttagactcaggcctggcccctg	68	219
16	F: ggcagggcacagcccctccatcg R: gtcaccctagccgccaccgccca	68	225
17	F: cccagcaggagccttgcccctga R: aagctgggaccctcccctcctgc	58	252
18	F: cccactggagaggtgtccattcc R: cccagccacatcatgggaatttaca	68	207
19	F: ccaactggagctgagcaggtggtc R: acacgtacacctgtatgtggctga	68	218
20	F: atccccaaacccactgtaccctg R: ctgggttcaaggcctgtttgggca	68	202
21	F: ggaatgggacagcaggtctgagc R: gaaactcctgcaccagggctcaga	68	318
22	F: ggtgggaatccctgcaacaacagc R: ggacaagccagcaagtgaccacta	68	223
23	F: agctgttccctgagcgtgtggca R: agcgtgtgtgtgggctctgga	68	323
24	F: gcttctcagtagctgggactcca R: tacggccaccaccagcagcagag	68	332
25	F: agcaatgtcctccgctctggcctc R: ctgtgcggcctgggagggacatca	68	270
26	F: ttgctttctgctcagccacttgacc R: ctgtgctccagcctaggccactca	67	206
27	F: gggaaaccccctaaactttacctgc R: aggcaccactcagccacaaaaga	67	236
28	F: gggctgttctctggtgggtgattcc R: ggcctagctccccttcccactca	67	223
29	F: gagggcctggcggctgccctca R: ctggggcactcagtgcaactgga	67	286
30	F: gtctgaagggagggcaccacaa R: caagttagtggcagcactggcagc	67	300
31	F: ccttccctgactctgtgcctgctc R: agcagggaggtgtgagccaaagca	67	325
32	F: ttggtggtgtggaaggcttctctg R: gttcaggtccacatcccttctctag	67	285
33*	F: caaactcaactcgtatgttctctctg R: gctggagctacagagcaagggac	58	218
34	F: cctgggtgtgggagcctgcctc R: cctctccttcccctctgtctgtc	67	222
35	F: cccactggttggggcatgactgac R: accctgaccccagtgccagggca	63	377
36	F: ctacgctgtctctgcccctatgg R: ctgagccgttaacctattgtacagctg	68	330
37	F: ccacaggtagagagctgacctgag R: accagacagtagcggagacctgca	68	238
38	F: tgccagcagtgggcgctgctga R: ggaggaaggcagtggtgcagacgaa	68	276
39	F: agtgcctcctctattcggcacaag R: cccgtcacagcaggtgaggtctg	68	252
40	F: tctctgactcccgatggcagctg R: atccccaaagggctcatcccacaa	68	294
41	F: gttccacagtcccacgcacatgc R: gccctgtcctgggcaactgcagac	68	201
42	F: gctcagtataggagcctatagccaga R: gttccgtaagttcctatggcccag	66	268
43 & 44	F: agctgccagacagggccttaggtgg R: agcaacgctagctgtgcacgaagg	66	505
45	F: tctgccaggtccctgcaagcctgt R: cctgggctgctggccaggtctctg	68	326
46*	F: ctggcctgcctgagcagggcctg R: atgcctgttcccctcctcctctg	58	228

TABLE 2. CONTINUED.

Exon	Primer sequence (5' to 3')	Annealing temperature (°C)	Amplicon size (bp)
47	F: cttgctctgggcccccatctgatg R: ctagagatagatgggtggctaggagg	66	235
48	F: agggcccaggccgtgctctctota R: tacgctgcaggcaaggcaggagc	66	216
49	F: tggccctgtcccaccgtgtgctc R: gaaccaactgctgctcgagagc	68	193

PCR products from all 48 coding exons of this gene were sequenced to identify the mutation in an affected individual. Once the mutation was identified, the rest of the family members were also examined for the presence of the mutation. * works with 10% DMSO. The forward (F) and reverse (R) primers are listed below.

RESULTS

All the five affected individuals had a classic USH1 phenotype which included congenital and profound sensorineural hearing loss with no vestibular response, and retinitis pigmentosa (RP). All five affected individuals started walking approximately at the age of five years. The age of the onset of RP was prepubertal in all affected individuals, which ranged from four to six years. All affected individuals had similar findings on examination with a small variation in vi-

sual acuity and visual fields (Table 3). All had excellent best corrected visual acuities ranging from 6/6p to 6/12. Visual fields (as determined by confrontational testing and Octopus automated perimetry) were restricted in all affected individuals. The extent of visual field ranged from 10° to 30°. The worst restriction was seen in the oldest affected individual IV-1. Anterior segment examination including the lens was normal in all affected individuals with normal pupillary reactions. Intraocular pressure measurements using Goldmann applanation tonometry were within normal limits for all affected individuals. Posterior segment findings were similar with all affected individuals showing features of central retinitis pigmentosa with arteriolar attenuation, disc pallor and tessellation of the fundus with bone spicule pigmentation in the posterior pole and equatorial fundus. A metallic sheen at the macula was seen in all affected individuals. However, macular function tests were normal in all affected individuals. The affected individual IV-4 had a longstanding total retinal detachment (probably secondary to trauma) in the left eye with only perception of light. Symptomatically, all five affected individuals complained of decreased night vision (nyctalopia). There were no other significant findings on neurological and other systemic examination.

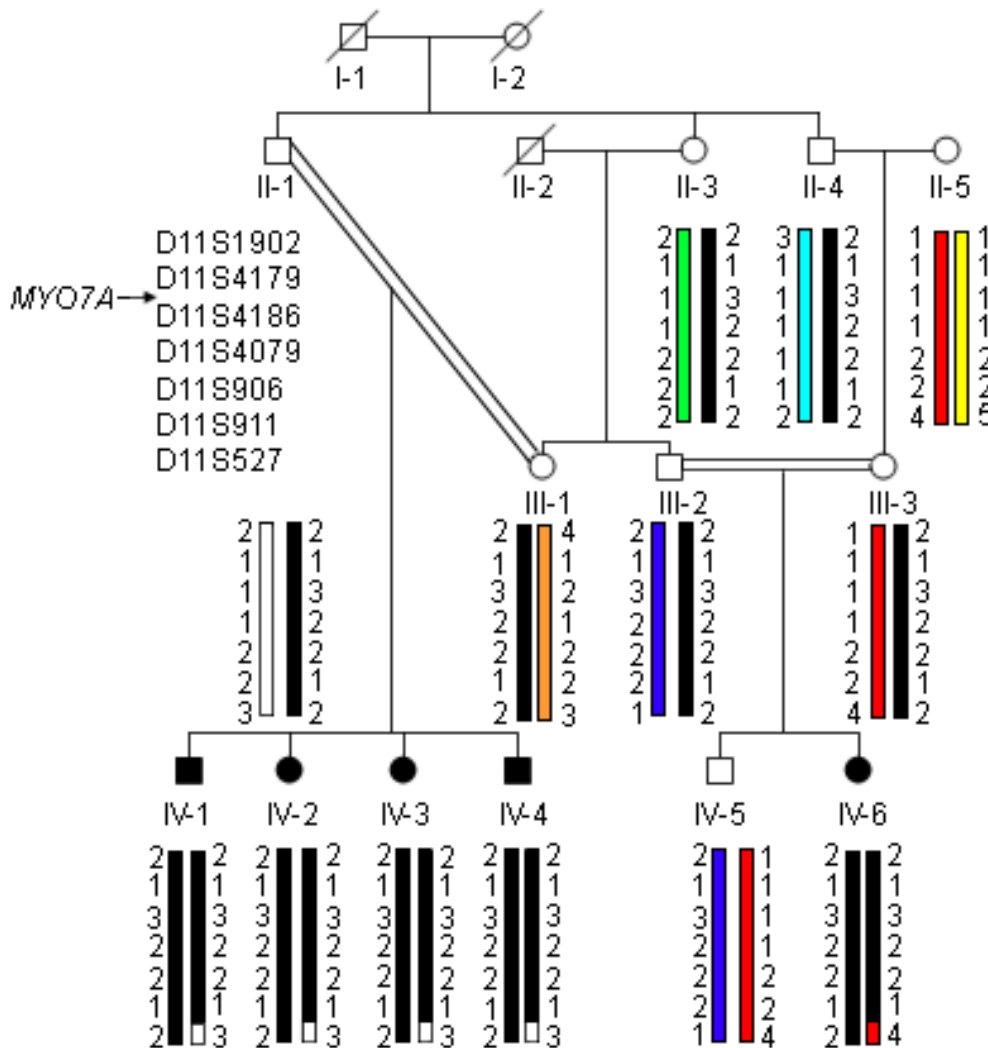


Figure 1. Haplotype analysis of the family with markers from the USH1B locus. Different haplotypes are shown by bars with different patterns. Black bar represents the disease haplotype. The position of the MYO7A gene is shown.

The visual inspection of the pedigree suggested an autosomal recessive mode of inheritance in the family (Figure 1). Haplotype analysis using markers selected from the candidate regions of all seven known USH1 loci suggested linkage of this family to the USH1B locus only (Figure 1). A disease haplotype 2-1-3-2-2-1 for markers D11S1902, D11S4179, D11S4186, D11S4079, D11S906 and D11S911 was co-segregating with the disease consistent with a homozygous state in all five affected individuals (Figure 1), suggesting that USH in this family is caused by a mutation in the *MYO7A* gene. DNA sequence analysis of entire coding region of this gene in an affected individual IV-4 showed an insertion of A residue between nucleotide positions 2663 and 2664 (c.2663_2664insA) in exon 22 in a homozygous state (Figure 2A), resulting in a premature stop codon in exon 23 (Figure 2B). PCR-SSCP analysis showed cosegregation of the mutation with the disease in the family (Figure 2C). This change was not present in 50 normal control individuals (data not shown).

DISCUSSION

Our review of the literature on the total number of mutations reported to date in the *MYO7A* gene responsible for USH

showed a total of 74 mutations with 48 being novel and 26 recurrent [13,14,27-35]. With the addition of the mutation reported in this study, the total number of known mutations in the *MYO7A* gene reaches to 75. In addition to USH causing mutations, four mutations in patients with autosomal recessive nonsyndromic deafness (DFNB2) [37,38] and four mutations in patients with autosomal dominant nonsyndromic deafness (DFNA11) [39-42] in the *MYO7A* gene have also been reported. Of 75 mutations that cause USH1B, 40 are missense, 16 are nonsense, 15 are deletions, and four are insertions. Although no mutations are detected in 12 of the 48 coding exons (exons 2, 10, 12, 20, 24, 26, 27, 32, 33, 34, 42, and 43), they are found to be scattered across the coding region, suggesting that mutation analysis in this gene will require evaluation of its complete coding region. The mutation, c.2663_2664insA in the present family is predicted to introduce a premature stop codon in exon 23 with a missense run of 18 amino acids starting from codon 889 in exon 22 (Figure 2B). The overall effect of this mutation is a premature truncation of MYO7A protein (Figure 2D). The mutated MYO7A protein is very unlikely to form dimers. In order to see if the insertion of the A residue has an affect on the splicing of the mutant *MYO7A* transcript, we carried out in silico analysis of putative exonic

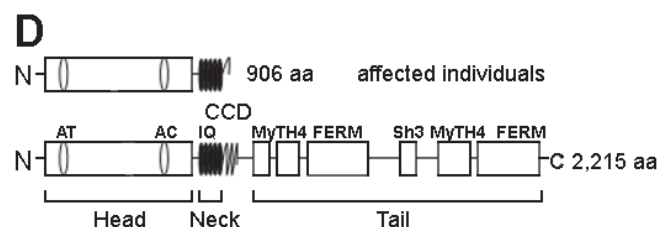
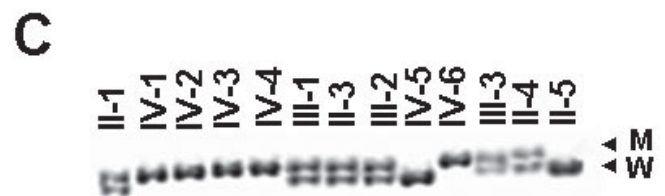
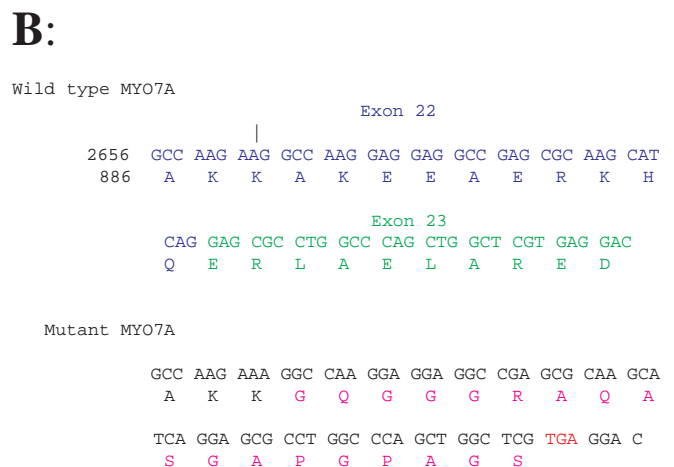
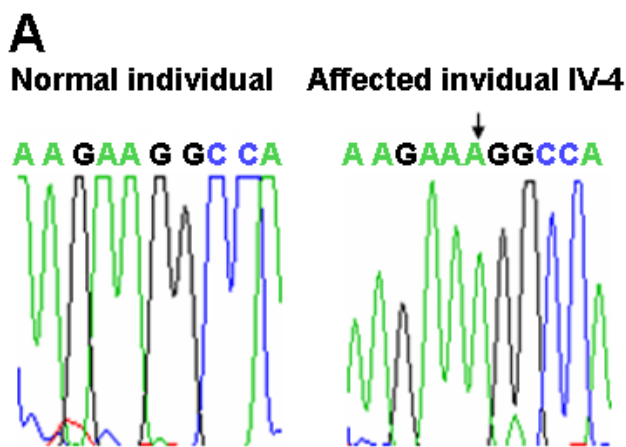


Figure 2. Mutation analysis of the *MYO7A* gene in the family. **A:** Sequencing chromatograms of PCR products of exon 22 from a normal individual and the affected individual IV-4; insertion of an A residue is marked by an arrow. **B:** Partial nucleotide and amino acid sequences of wild type (normal) and mutant alleles; insertion site of an A residue is marked above by a “pipe” (|) in the wild type allele; a missense run of 18 amino acids are shown in purple in the mutant protein; premature stop codon in exon 23 is shown in red. **C:** PCR-SSCP analysis of all 13 individuals from the family, note all five affected individuals (IV-1, IV-2, IV-3, IV-4, and IV-6) are homozygous for the mutant allele (M) and their parents (II-1, III-1, III-2, and III-3) are heterozygous for the mutant and the wild type (W) alleles. **D:** Schematic representation of mutant (above) and normal MYO7A (below) proteins. Note the truncation of MYO7A protein in affected individuals. The normal protein has a head domain with an ATP binding site (AT) and an actin binding site (AC), followed by a neck region with isoleucine glutamine motifs (IQ), a short coiled-coil domain (CCD), and a tail region with myosin tail homology 4 (MyTH4) domain, 4.1 protein ezerin-radixin-moesin-like domain (FERM) and src homology type 3 (SH3) domain.

splicing enhancers and control elements in the normal and the mutant alleles using the RESCUE-ESE program [43]. The analysis of the normal allele predicted three enhancers, CAAGAA, AAGAAG, and AGAAGG at the site of insertion in the normal allele. Whereas, it predicted one common, CAAGAA, and two different, AAGAAA and AGAAAG, enhancers in the mutant allele at the site of insertion (Figure 3). This suggests that the alteration of enhancers at the insertion site may influence splicing of the mutant *MYO7A* transcript in patients, although it remains to be experimentally proven.

The human *MYO7A* gene consists of 49 exons, the first being a noncoding one, and produces a 7.5 kb transcript [30]. The *MYO7A* gene is expressed in cochlea and retina [44]. It encodes a protein of 2,215 amino acids (230 kDa) [28]. *MYO7A* protein contains an N-terminal head domain with ATP and actin binding sites followed by a 126 amino acid neck domain containing five IQ motifs serving as binding sites for calmodulin, a short 78 amino acid long coiled-coil domain for dimerization and a C-terminal tail region [37]. As a result of the insertion mutation, *MYO7A* mutant protein is predicted to contain only 906 amino acids with last 18 amino acids being different (Figure 2D). This mutant protein lacks the tail region. The tail domain of *MYO7A* is believed to be largely responsible for its function [45]. Recently, several proteins, such as MAP2B, RI α of PKA, Keap1, vezatin, MyRIP, and harmonin, have been identified that interact with different domains in the tail region of *MYO7A* [46-51], indicating that these proteins specify the cellular function of *MYO7A*. Further, *MYO7A* protein has been suggested to be targeted to the plasma membrane by its FERM domains in the tail region [52]. Recently, it has been shown that *MYO7A* is a component of a supramolecular USH1 complex integrated in the plasma membrane via harmonin [51]. The scaffold protein harmonin binds the *MYO7A* tail via its PDZ domain 1 [51]. The supramolecular USH1 complex is necessary for the proper differentiation of the stereovilli in the mechanosensitive hair cells [51]. Taken together, the absence of the tail region of mutant *MYO7A* protein will abrogate its function, resulting in the disease phenotype in patients reported here.

Wild type *MYO7A* allele

AGAAGG
AAGAAG
CAAGAA
2656 GCCAAGAAGGCCAAG

Mutant *MYO7A* allele

AGAAAG
AAGAAA
CAAGAA
2656 GCCAAGAAAGGCCAAG

Figure 3. In silico analysis of putative exon enhancers. cDNA sequences of the wild type and mutant alleles are shown. Putative exon enhancers are shown above cDNA sequences in both alleles. The number represents the nucleotide position in the cDNA sequence.

In summary, we have shown our results on the genetic analysis of a four generation Indian family with USH1. Our analysis suggested linkage of this family to the *USH1B* locus on chromosome 11q. Mutation analysis of the *MYO7A* gene revealed a novel insertion mutation in this family, resulting in premature truncation of the protein and disease phenotype in patients. Our review of the literature showed that the total number of USH causing mutations in the *MYO7A* gene reaches to 75. The information presented here will be useful to provide prenatal diagnosis and genetic counseling to the family and their relatives.

ACKNOWLEDGEMENTS

We are thankful to the members of the family for their participation in this study. This work was supported by grants from the University Grants Commission and the Department of Science and Technology, New Delhi.

REFERENCES

1. Usher CH. On the inheritance of retinitis pigmentosa, with notes of cases. R Lond Ophthalmol Hosp Rep 1914; 19:130-236.
2. Smith RJ, Berlin CI, Hejtmancik JF, Keats BJ, Kimberling WJ, Lewis RA, Moller CG, Pelias MZ, Tranebjaerg L. Clinical diagnosis of the Usher syndromes. Usher Syndrome Consortium. Am J Med Genet 1994; 50:32-8.
3. Grondahl J. Estimation of prognosis and prevalence of retinitis pigmentosa and Usher syndrome in Norway. Clin Genet 1987; 31:255-64.
4. Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. J Chronic Dis 1983; 36:595-603.
5. Astuto LM, Weston MD, Carney CA, Hoover DM, Cremers CW, Wagenaar M, Moller C, Smith RJ, Pieke-Dahl S, Greenberg J, Ramesar R, Jacobson SG, Ayuso C, Heckenlively JR, Tamayo M, Gorin MB, Reardon W, Kimberling WJ. Genetic heterogeneity of Usher syndrome: analysis of 151 families with Usher type I. Am J Hum Genet 2000; 67:1569-74.
6. Kaplan J, Gerber S, Bonneau D, Rozet JM, Delrieu O, Briard ML, Dollfus H, Ghazi I, Dufier JL, Frezal J, Munnich A. A gene for Usher syndrome type I (*USH1A*) maps to chromosome 14q. Genomics 1992; 14:979-87.
7. Kimberling WJ, Moller CG, Davenport S, Priluck IA, Beighton PH, Greenberg J, Reardon W, Weston MD, Kenyon JB, Grunkemeyer JA, Pieke Dahl S, Overbeck LD, Blackwood DJ, Brower AM, Hoover DM, Rowland P, Smith RJH. Linkage of Usher syndrome type I gene (*USH1B*) to the long arm of chromosome 11. Genomics 1992; 14:988-94.
8. Smith RJH, Lee EC, Kimberling WJ, Daiger SP, Pelias MZ, Keats BJB, Jay M, Bird A, Reardon W, Guest M, Ayyagari R, Hejtmancik JF. Localization of two genes for Usher syndrome type I to chromosome 11. Genomics 1992; 14:995-1002.
9. Wayne S, Der Kaloustian VM, Schloss M, Polomeno R, Scott DA, Hejtmancik JF, Sheffield VC, Smith RJ. Localization of the Usher syndrome type 1D gene (*Ush1D*) to chromosome 10. Hum Mol Genet 1996; 5:1689-92.
10. Chaib H, Kaplan J, Gerber S, Vincent C, Ayadi H, Slim R, Munnich A, Weissenbach J, Petit C. A newly identified locus for Usher syndrome type I, *USH1E*, maps to chromosome 21q21. Hum Mol Genet 1997; 6:27-31.
11. Wayne S, Lowry RB, McLeod DR, Knaus R, Farr C, Smith RJH.

- Localization of the Usher syndrome type 1F to chromosome 10. *Am J Hum Genet* 1997; 61:A300.
12. Mustapha M, Chouery E, Torchard-Pagnez D, Nouaille S, Khraiss A, Sayegh FN, Megarbane A, Loiselet J, Lathrop M, Petit C, Weil D. A novel locus for Usher syndrome type I, USH1G, maps to chromosome 17q24-25. *Hum Genet* 2002; 110:348-50.
 13. Adato A, Weil D, Kalinski H, Pel-Or Y, Ayadi H, Petit C, Korostishevsky M, Bonne-Tamir B. Mutation profile of all 49 exons of the human myosin VIIA gene, and haplotype analysis, in Usher 1B families from diverse origins. *Am J Hum Genet* 1997; 61:813-21.
 14. Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, Mburu P, Varela A, Leveilliers J, Weston MD, Kelley PM, Kimberling WJ, Wagenaar M, Levi-Acobas F, Larget-Piet D, Munnich A, Steel KP, Brown SDM, Petit C. Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* 1995; 374:60-1.
 15. Liu XZ, Hope C, Walsh J, Newton V, Ke XM, Liang CY, Xu LR, Zhou JM, Trump D, Steel KP, Bunday S, Brown SD. Mutations in the myosin VIIA gene cause a wide phenotypic spectrum, including atypical Usher syndrome. *Am J Hum Genet* 1998; 63:909-12.
 16. Verpy E, Leibovici M, Zwaenepoel I, Liu XZ, Gal A, Salem N, Mansour A, Blanchard S, Kobayashi I, Keats BJ, Slim R, Petit C. A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C. *Nat Genet* 2000; 26:51-5.
 17. Bolz H, von Brederlow B, Ramirez A, Bryda EC, Kutsche K, Nothwang HG, Seeliger M, del C-Salcedo Cabrera M, Vila MC, Molina OP, Gal A, Kubisch C. Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. *Nat Genet* 2001; 27:108-12.
 18. Alagramam KN, Yuan H, Kuehn MH, Murcia CL, Wayne S, Srisailpathy CR, Lowry RB, Knaus R, Van Laer L, Bernier FP, Schwartz S, Lee C, Morton CC, Mullins RF, Ramesh A, Van Camp G, Hageman GS, Woychik RP, Smith RJ, Hageman GS. Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F. *Hum Mol Genet* 2001; 10:1709-18. Erratum in: *Hum Mol Genet* 2001; 10:2603.
 19. Weil D, El-Amraoui A, Masmoudi S, Mustapha M, Kikkawa Y, Laine S, Delmaghani S, Adato A, Nadifi S, Zina ZB, Hamel C, Gal A, Ayadi H, Yonekawa H, Petit C. Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. *Hum Mol Genet* 2003; 12:463-71.
 20. Kimberling WJ, Weston MD, Moller C, Davenport SL, Shugart YY, Priluck IA, Martini A, Milani M, Smith RJ. Localization of Usher syndrome type II to chromosome 1q. *Genomics* 1990; 7:245-9.
 21. Hmani M, Ghorbel A, Boulila-Elgaied A, Ben Zina Z, Kammoun W, Drira M, Chaabouni M, Petit C, Ayadi H. A novel locus for Usher syndrome type II, USH2B, maps to chromosome 3 at p23-24.2. *Eur J Hum Genet* 1999; 7:363-7.
 22. Pieke-Dahl S, Moller CG, Kelley PM, Astuto LM, Cremers CW, Gorin MB, Kimberling WJ. Genetic heterogeneity of Usher syndrome type II: localisation to chromosome 5q. *J Med Genet* 2000; 37:256-62.
 23. Weston MD, Eudy JD, Fujita S, Yao S, Usami S, Cremers C, Greenberg J, Ramesar R, Martini A, Moller C, Smith RJ, Sumegi J, Kimberling WJ, Greenburg J. Genomic structure and identification of novel mutations in usherin, the gene responsible for Usher syndrome type IIa. *Am J Hum Genet* 2000; 66:1199-210. Erratum in: *Am J Hum Genet* 2000; 66:2020.
 24. Weston MD, Luijendijk MW, Humphrey KD, Moller C, Kimberling WJ. Mutations in the VLRG1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. *Am J Hum Genet* 2004; 74:357-66. Erratum in: *Am J Hum Genet*. 2004; 74:1080.
 25. Sankila EM, Pakarinen L, Kaariainen H, Aittomaki K, Karjalainen S, Sistonen P, de la Chapelle A. Assignment of an Usher syndrome type III (USH3) gene to chromosome 3q. *Hum Mol Genet* 1995; 4:93-8.
 26. Joensuu T, Hamalainen R, Yuan B, Johnson C, Tegelberg S, Gasparini P, Zelante L, Pirvola U, Pakarinen L, Lehesjoki AE, de la Chapelle A, Sankila EM. Mutations in a novel gene with transmembrane domains underlie Usher syndrome type 3. *Am J Hum Genet* 2001; 69:673-84. Erratum in: *Am J Hum Genet* 2001; 69:1160.
 27. Weston MD, Kelley PM, Overbeck LD, Wagenaar M, Orten DJ, Hasson T, Chen ZY, Corey D, Mooseker M, Sumegi J, Cremers C, Moller C, Jacobson SG, Gorin MB, Kimberling WJ. Myosin VIIA mutation screening in 189 Usher syndrome type I patients. *Am J Hum Genet* 1996; 59:1074-83.
 28. Levy G, Levi-Acobas F, Blanchard S, Gerber S, Larget-Piet D, Chenal V, Liu XZ, Newton V, Steel KP, Brown SD, Munnich A, Kaplan J, Petit C, Weil D. Myosin VIIA gene: heterogeneity of the mutations responsible for Usher syndrome type IB. *Hum Mol Genet* 1997; 6:111-6.
 29. Liu XZ, Newton VE, Steel KP, Brown SD. Identification of a new mutation of the myosin VII head region in Usher syndrome type I. *Hum Mutat* 1997; 10:168-70.
 30. Janecke AR, Meins M, Sadeghi M, Grundmann K, Apfelstedt-Sylla E, Zrenner E, Rosenberg T, Gal A. Twelve novel myosin VIIA mutations in 34 patients with Usher syndrome type I: confirmation of genetic heterogeneity. *Hum Mutat* 1999; 13:133-40.
 31. Cuevas JM, Espinos C, Millan JM, Sanchez F, Trujillo MJ, Ayuso C, Beneyto M, Najera C. Identification of three novel mutations in the MYO7A gene. *Hum Mutat* 1999; 14:181.
 32. Cuevas JM, Espinos C, Millan JM, Sanchez F, Trujillo MJ, Garcia-Sandoval B, Ayuso C, Najera C, Beneyto M. Detection of a novel Cys628STOP mutation of the myosin VIIA gene in Usher syndrome type Ib. *Mol Cell Probes* 1998; 12:417-20.
 33. Bharadwaj AK, Kasztejna JP, Huq S, Berson EL, Dryja TP. Evaluation of the myosin VIIA gene and visual function in patients with Usher syndrome type I. *Exp Eye Res* 2000; 71:173-81.
 34. Espinos C, Millan JM, Sanchez F, Beneyto M, Najera C. Ala397Asp mutation of myosin VIIA gene segregating in a Spanish family with type-Ib Usher syndrome. *Hum Genet* 1998; 102:691-4.
 35. Najera C, Beneyto M, Blanca J, Aller E, Fontcuberta A, Millan JM, Ayuso C. Mutations in myosin VIIA (MYO7A) and usherin (USH2A) in Spanish patients with Usher syndrome types I and II, respectively. *Hum Mutat* 2002; 20:76-7.
 36. Kumar A, Kandt RS, Wolpert C, Roses AD, Pericak-Vance MA, Gilbert JR. Mutation analysis of the TSC2 gene in an African-American family. *Hum Mol Genet* 1995; 4:2295-8.
 37. Weil D, Kussel P, Blanchard S, Levy G, Levi-Acobas F, Drira M, Ayadi H, Petit C. The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nat Genet* 1997; 16:191-3.
 38. Liu XZ, Walsh J, Mburu P, Kendrick-Jones J, Cope MJ, Steel KP, Brown SD. Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nat Genet* 1997; 16:188-90.
 39. Liu XZ, Walsh J, Tamagawa Y, Kitamura K, Nishizawa M, Steel KP, Brown SD. Autosomal dominant non-syndromic deafness

- caused by a mutation in the myosin VIIA gene. *Nat Genet* 1997; 17:268-9.
40. Bolz H, Bolz SS, Schade G, Kothe C, Mohrmann G, Hess M, Gal A. Impaired calmodulin binding of myosin-7A causes autosomal dominant hearing loss (DFNA11). *Hum Mutat* 2004; 24:274-5.
 41. Luijendijk MW, Van Wijk E, Bischoff AM, Krieger E, Huygen PL, Pennings RJ, Brunner HG, Cremers CW, Cremers FP, Kremer H. Identification and molecular modelling of a mutation in the motor head domain of myosin VIIA in a family with autosomal dominant hearing impairment (DFNA11). *Hum Genet* 2004; 115:149-56.
 42. Street VA, Kallman JC, Kiemele KL. Modifier controls severity of a novel dominant low-frequency MyosinVIIA (MYO7A) auditory mutation. *J Med Genet* 2004; 41:e62.
 43. Fairbrother WG, Yeo GW, Yeh R, Goldstein P, Mawson M, Sharp PA, Burge CB. RESCUE-ESE identifies candidate exonic splicing enhancers in vertebrate exons. *Nucleic Acids Res* 2004; 32:W187-90.
 44. Hasson T, Heintzelman MB, Santos-Sacchi J, Corey DP, Mooseker MS. Expression in cochlea and retina of myosin VIIa, the gene product defective in Usher syndrome type 1B. *Proc Natl Acad Sci U S A* 1995; 92:9815-9.
 45. Oliver TN, Berg JS, Cheney RE. Tails of unconventional myosins. *Cell Mol Life Sci* 1999; 56:243-57.
 46. Todorov PT, Hardisty RE, Brown SD. Myosin VIIA is specifically associated with calmodulin and microtubule-associated protein-2B (MAP-2B). *Biochem J* 2001; 354:267-74.
 47. Kussel-Andermann P, El-Amraoui A, Safieddine S, Nouaille S, Perfettini I, Lecuit M, Cossart P, Wolfrum U, Petit C. Vezatin, a novel transmembrane protein, bridges myosin VIIA to the cadherin-catenins complex. *EMBO J* 2000; 19:6020-9.
 48. Kussel-Andermann P, El-Amraoui A, Safieddine S, Hardelin JP, Nouaille S, Camonis J, Petit C. Unconventional myosin VIIA is a novel A-kinase-anchoring protein. *J Biol Chem* 2000; 275:29654-9.
 49. Velichkova M, Guttman J, Warren C, Eng L, Kline K, Vogl AW, Hasson T. A human homologue of *Drosophila* kelch associates with myosin-VIIa in specialized adhesion junctions. *Cell Motil Cytoskeleton* 2002; 51:147-64.
 50. El-Amraoui A, Schonn JS, Kussel-Andermann P, Blanchard S, Desnos C, Henry JP, Wolfrum U, Darchen F, Petit C. MyRIP, a novel Rab effector, enables myosin VIIa recruitment to retinal melanosomes. *EMBO Rep* 2002; 3:463-70.
 51. Boeda B, El-Amraoui A, Bahloul A, Goodyear R, Daviet L, Blanchard S, Perfettini I, Fath KR, Shorte S, Reiners J, Houdusse A, Legrain P, Wolfrum U, Richardson G, Petit C. Myosin VIIa, harmonin and cadherin 23, three Usher I gene products that cooperate to shape the sensory hair cell bundle. *EMBO J* 2002; 21:6689-99.
 52. Chishti AH, Kim AC, Marfatia SM, Lutchman M, Hanspal M, Jindal H, Liu SC, Low PS, Rouleau GA, Mohandas N, Chasis JA, Conboy JG, Gascard P, Takakuwa Y, Huang SC, Benz EJ Jr, Bretscher A, Fehon RG, Gusella JF, Ramesh V, Solomon F, Marchesi VT, Tsukita S, Tsukita S, Hoover KB. The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane. *Trends Biochem Sci* 1998; 23:281-2.