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Myosin VIIA Mutation Screening in 189 Usher Syndrome Type 1 Patients

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Summary

Usher syndrome type 1b (USH1B) is an autosomal recessive disorder characterized by congenital profound hearing loss, vestibular abnormalities, and retinitis pigmentosa. The disorder has recently been shown to be caused by mutations in the myosin VIIa gene (MYO7A) located on 11q14. In the current study, a panel of 189 genetically independent Usher I cases were screened for the presence of mutations in the N-terminal coding portion of the motor domain of MYO7A by heteroduplex analysis of 14 exons. Twenty-three mutations were found segregating with the disease in 20 families. Of the 23 mutations, 13 were unique, and 2 of the 13 unique mutations (Arg212His and Arg212Cys) accounted for the greatest percentage of observed mutant alleles (8/23, 31%). Six of the 13 mutations caused premature stop codons, 6 caused changes in the amino acid sequence of the myosin VIIa protein, and 1 resulted in a splicing defect. Three patients were homozygotes or compound heterozygotes for mutant alleles; these three cases were Tyr333Stop/Tyr333Stop, Arg212His-Arg302His/Arg212His-Arg302His, and IVS13nt-8c→g/Glu450Gln. All the other USH1B mutations observed were simple heterozygotes, and it is presumed that the mutation on the other allele is present in the unscreened regions of the gene. None of the mutations reported here were observed in 96 unrelated control samples, although several polymorphisms were detected. These results add three patients to single case reported previously where mutations have been found in both alleles and raises the total number of unique mutations in MYO7A to 16.

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Introduction

Phenotypically, there are three types of Usher syndrome: I, II, and III. Type I has a profound congenital hearing loss, vestibular areflexia, and retinitis pigmentosa (RP). Type II has a mild hearing loss in the low frequencies, sloping to severe in the high frequencies, normal vestibular responses, and RP. Type III has a progressive loss, variable vestibular problems, and RP. At least three different genes are responsible for the more severe type I Usher syndrome. These genes are located on 11q (USH1B [Kimberling et al. 1992]), 11p (USH1C [Smith et al. 1992]), and 14q (USH1A [Kaplan et al. 1992]). There are no known clinical differences between these three subtypes of Usher type I, and they can be distinguished only by linkage in informative families. Usher Ib is the most common subtype, accounting for 70%–80% of all type I cases. Usher Ia accounts for most of the other cases, while Usher Ic has been observed exclusively in people with French Acadian ancestry.

The Usher syndrome type 1b (USH1B) subtype has been localized to 11q14 (Kimberling et al. 1992). Recently, a 3.5-kb partial cDNA from human testis coding for myosin VIIa (MYO7A) has been cloned from this region (Hasson et al. 1995). Deleterious mutations within the coding sequence are associated with families segregating the disorder (Weil et al. 1995). Similarly, mutations in the homologous mouse myo7 gene cause the shaker-1 (*sh-1*), whose phenotype is deafness without retinal dystrophy (Gibson et al. 1995). DFNB2 is a human neurosensorial recessive deafness gene that is unassociated with retinal degeneration but maps to the same location on 11q as USH1B (Guilford et al. 1994), raising the possibility that some mutations in MYO7A may cause a human phenotypic equivalent of *sh-1*.

We undertook a mutation screening study of type I Usher cases by heteroduplex analysis (HA) of 14 exons to gain insights into the position and nature of different MYO7A mutations and to lay a foundation for studies

into whether different mutations result in different phenotypes.

Usher syndrome is responsible for ~5% of all congenital deafness (Kimberling and Möller 1995), and a method of rapid and inexpensive screening of young patients with congenital profound deafness would be beneficial. Early identification of Usher cases will affect a child's education as well as enhance his/her safety by alerting parents about potential problems with night and peripheral vision. In order to evaluate different strategies for such large-scale mutation screening, the frequency of specific myosin-VIIa mutations and their distribution relative to different populations and ethnic groups must be determined.

This is a report on 13 new MYO7A mutations that were observed exclusively in a panel of Usher Ib patients. The nature of each mutation and the predicted mechanisms for MYO7A inactivation is discussed.

Subjects and Methods

Subjects

The cases studied here were collected as part of a long-standing study on the genetics of Usher syndrome. Patients were considered affected with Usher type I, on the following basis: congenital severe to profound hearing loss, vestibular areflexia, and RP (confirmed in at least one affected family member by an electroretinogram). The cases studied were generally of two types; informative families compatible with linkage to 11q14 markers, which are presumed to have Usher Ib, or singleton cases with a phenotype fitting the diagnostic criteria stated above. A linkage diagnosis was not possible for the singleton cases; therefore, we are not absolutely certain that they are Usher type Ib. However, data on the frequency of linked families indicates that >80% of all patients with type I Usher syndrome are linked to 11q and are presumed to carry MYO7A mutations. Usher I families failing to link to 11q markers flanking the USH1B locus were not studied.

7D11 DNA

A cosmid array produced from the partial *Sau*3AI digestion of CEPH YAC 802a5 (P. M. Kelley, unpublished results) was screened using a 4.6-kb mouse cDNA, ET58 (Gibson et al. 1995). Cosmid 7D11 was identified and a 4.2-kb and a 6.0-kb *Eco*RI fragments hybridizing to ET58 were subcloned. The sequence was obtained by generating deletion clones using the Erase-a-Base System® (Promega) and primer walking using oligonucleotides synthesized on a Cruachem PS250. These subclones contained the 10 exons from the MYO7A head region reported by Weil et al. (1995).

Intron/Exon boundaries of 4 additional 5′ exons were identified by sequencing 7D11 template using primers designed from the sequence of MYO7A cDNA (Hasson et al. 1995). These 14 exons correspond to the first 1,957 bases of the cDNA (Accession number U34227), and the sequence information was used to design the primers for mutation screening.

Mutation Detection

Intronic PCR amplification primers flanking each exon (table 1) were used for screening mutations by HA (Keen et al. 1991). The exceptions were exons 4 and 9. For each of these exons, one primer spanned an intron/exon boundary. PCR reaction mixtures contained 200 ng of genomic DNA, 100 pmol of each exon-specific primer pair, 1.5 mM $MgCl_2$, and dNTPs (200 μM) in a total volume of 100 μl. Amplification was carried out using a hot-start method to minimize false priming: 96°C for 5 min, followed by a soak at 80°C, then 2.5 U of *Taq* DNA polymerase (Perkin Elmer) and dNTPs were added followed by 37 cycles of 30 s at 95°C, 30 s at 60°C, and 45 s at 72°C, with a final incubation for 5 min at 72°C.

Homozygous and heterozygous mutations were detected as follows: PCR products amplified from genomic USH1B samples with 7D11 cosmid were mixed, heated at 95°C for 3 min, and cooled to 37°C over a 20–30-min period. The reannealed reaction products were electrophoresed through 35-cm × 43-cm × 1-mm 1× MDE™ gels (FMC) at 850 V for 16–24 h. PCR reactions were repeated on the heteroduplex positive samples and all available family members. HA was done both with and without 7D11 PCR control, to determine whether the patient was homozygous or heterozygous. Genomic DNA from a set of 96 normal genetically independent control samples were also analyzed to help differentiate pathologic mutations from probable polymorphisms.

Amplified exons from patients were purified by the Wizard™ PCR clean-up kit (Promega) and sequenced using the fmol® sequencing kit (Promega) using appropriate end-labeled primers. If direct sequencing did not unequivocally identify the DNA sequence change, the PCR product was cloned into a plasmid vector (AT cloning kit, Invitrogen) to separate individual alleles prior to sequencing.

Results

Of 378 USH1B chromosomes surveyed for mutations by HA, 13 different mutations were found in a total of 23 USH1B chromosomes from 20 probands. Table 2 summarizes the mutations observed. Codon numbering starts with the first in-frame methionine of MYO7A (Hasson et al. 1995; Weil et al. 1996; Chen et al., in press). Figure 1 shows the relative locations of each mutation along a linear representation of the protein.

Table 1

MYO7A PCR Primers

Exon and Primer	Sequence	PCR Fragment Size (bp)
1:		
MYO7-1U	GAG·CAG·CCT·CTG·GAC·ATT·AGT·C	441
MYO7-1L	CCA·TGA·CTC·CTC·CAC·ATC·CA	
2:		
MYO7-2U	CAG·AAG·CAT·GAC·ATG·GTC·TCT·CT	206
MYO7-2L	GCA·GGA·ATT·TTC·CAA·GAG·AAC·AC	
3:		
MYO7-3U	CCT·TGT·GTG·GTT·GGC·TCA·GA	312
MYO7-3L	TCG·ATC·ATC·TCC·CTG·CAT·CA	
4:		
MYO7-4U	ACC·ATC·CTG·ACT·GAA·CCC·TGT	328
MYO7-4L	TCA·CGT·AGA·TGA·GGT·GGT·CC	
5:		
MYO7-5U	AGT·CAG·AGT·CTC·CCA·CAT·GGA	310
MYO7-5L	ATC·AGC·AGG·AGC·GCA·CAG·T	
6:		
MYO7-6U	TGT·GGG·TTG·TGA·CAG·GTC·CT	241
MYO7-6L	TGC·GGA·GTG·AGT·AGG·GTG·TC	
7:		
MYO7-7U	ACC·AGA·GTT·CCG·AGG·GTG·	260
MYO7-7L	AGG·GGC·CTG·GGT·CTA·TTC·	
8:		
MYO7-8U	CAC·TGT·GCC·CAC·ATT·TTC·AG	195
MYO7-8L	GCC·AGC·TCC·CTA·CAA·TCC·TA	
9:		
MYO7-9U	CCA·CCA·GGG·TAA·CTG·CAT·AAC	272
MYO7-9L	AAG·AGG·GCA·CGT·GCT·AGG·A	
10:		
MYO7-10U	CCT·GGG·GAA·GCA·TTT·AGT·CA	354
MYO7-10L	TGA·ACA·GAA·CTC·TCC·AAA·CCA·GA	
11:		
MYO7-11U	GGG·CTG·TCA·AGG·AGA·GAG·AAG	393
MYO7-11L	CCC·ATC·AAG·AGC·CCA·ACT·TC	
12:		
MYO7-12U	GGT·TTC·ACA·CGG·CAC·TTT·G	365
MYO7-12L	GCC·AGG·AGC·CAA·CTA·AAT·GTG	
13:		
MYO7-13U	GGA·GGG·AGG·TGG·ACT·TGA·C	432
MYO7-13L	AAA·GCA·GGG·AAG·GAA·GCT·GT	
14:		
MYO7-14U	CCA·GAG·AGC·GCC·TAT·GTG·AG	349
MYO7-14L	CTT·ATT·CCT·GGG·CTG·GAG·CA	

Missense Mutations

Six different missense mutations were found in 13 Usher I patients. Seven Usher 1b patients were observed with missense mutations in exon 7. These are the Arg212His and Arg212Cys mutations originally observed in two Usher 1b cases as heterozygotes (Weil et al. 1995). They are believed to be pathologic because (1) they occur within an evolutionarily conserved heptapeptide sequence that is invariant in all myosins studied and (2) neither has been observed in a series of control persons. Figure 2 shows the heteroduplex pattern observed in two Usher Ib families with the Arg212His mutation. PCR sequencing of exon 7 was carried out on at least one heteroduplex positive patient in each family to verify that the mutation was due to either a G→A(CGT→CAT) or a C→T(CGT→TGT) transition in codon 212. Furthermore, an *Alu*I site is created by the Arg212Cys (C→T) mutation. Amplified exon 7 fragments were digested with *Alu*I, and all Arg212Cys (C→T) mutations showed the predicted restriction fragment sizes following agarose gel electrophoresis.

Table 2

Summary of Mutations in 189 Usher-Type IB Cases for MYO7A Exons 1–14

Patient Status and Codon Change	Exon	Mutation	No. of Chromosomes	Origin
Heterozygotes:				
Cys31Stop	3	TGC→TGA	1	Sweden
			1	Holland
75delG(FS)	4	del GAC	1	Australia
120delC(FS)	5	del CGCCAG	1	Holland
Arg212His	7	CGT→CAT	1	USA
Arg212Cys	7	CGT→TGT	2	USA
			1	Holland
			1	Belgium[a]
Arg302His	9	CGC→CAC	1	Holland
			1	Sweden
Glu314Stop	9	GAG→TAG	1	USA
Arg212His-Arg302His	7 and 9	See above	1	Holland
468+Gln	13	insert GCA	2	USA
Pro503Leu	13	CCC→CTC	1	USA
532delA(FS)	14	del CAC	1	USA
Homozygotes:				
Arg212His-Arg302His	7 and 9	See above	2	Belgium[a]
Tyr333Stop	9	TAT→TAG	2	USA
Compound Heterozygote:				
Glu450Gln/	13 and	GAG→CAG	1	USA
IVS13nt-8c→g	14	ctccccag→gtccccag	1	

[a] These families were collected from the West-Flanders region, a Dutch-speaking part of Belgium.

Figure 2 also shows the heteroduplex pattern of a Arg212His mutation (exon 7) in *cis* with an Arg302His mutation (exon 9) in two Usher Ib families. Affected siblings in a Dutch family (1515) are homozygous for a double mutation at both codons, while the affected siblings in Flemish family (1062) show only paternal inheritance of both mutations. Arg302His and Arg212His have both been observed singly in affected persons. Neither of the two mutations were observed in controls either singly or as double mutations.

Three exon 13 mutations were observed. Figure 3*A* shows the segregation of the heteroduplex pattern caused by a GCA insertion at codon 468 (468+Gln) in family 985. This three-base insertion was also observed in one additional singleton case as a heterozygote. The exon 13 mutation Pro503Leu was observed in family 734 (Fig. 3*B*). This is an unusual family because the proband also has Crouzon syndrome. His sister, who carries the Pro503Leu mutation, was reported to have a progressive bilateral hearing loss but does not have RP. The third exon 13 mutation occurred in trans with a splice-site mutation and is explained in the following paragraph.

Splice Site Mutation

A C→G transversion at position −8 in the intronic splice-acceptor region preceding exon 14 was observed in one family, 218 (fig. 3*B*). The predicted effect of such a mutation is the disruption of the polypyrimidine tract characteristic of the majority of vertebrate 3′ splice sites. Normal splicing would be inhibited by impaired binding of various splicosome components that interact with the polypyrimidine tract (Krawczak et al. 1992). The proband from this family also inherited a G→C transversion in exon 13 at codon 450 (Glu450Gln) from the other parent (fig. 3*B*). The contribution of mutant alleles from each parent in this family is consistent with the hypothesis that the presence of both mutated alleles in the proband is responsible for the Usher type I phenotype.

Nonsense Mutations

Six mutations that directly or indirectly, through a frameshift, lead to premature stop codons were found in seven Usher Ib cases. Two unrelated cases (one from Sweden and the other from the United States) were found to be heterozygous for a C→A transversion in exon 3 at codon 31, causing a Cys→Stop, (TGC→TGA). The U.S. family has Scandinavian ancestry, and so one must consider the possibility that the two are derived from the same ancestral mutation.

A deletion of a single G in a GGGGG string in exon 4 causes a frameshift starting a codon 75 and continues for 30 amino acids and ends at a UGA stop codon. This

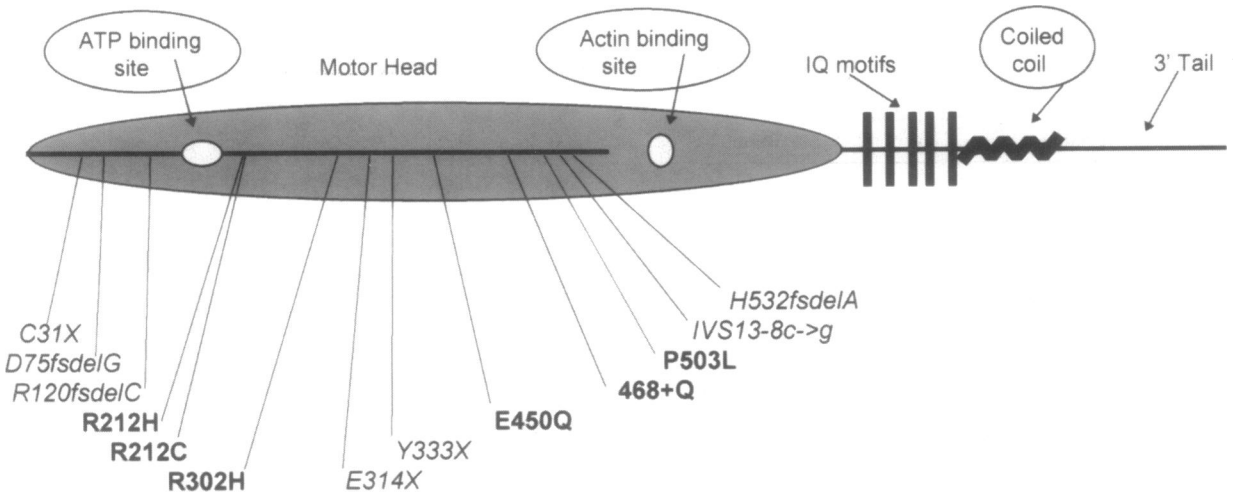

Figure 1 Partial domain structure of myosin VIIa protein with relevant motifs, which define the myosin superfamily of proteins. The 3′ tail domains specific to myosin VIIa are omitted from this figure. The relative linear location of each of the mutations are shown. Boldfaced mutations are missense, and italicized mutations are frameshift, nonsense, and splice-site mutations. The horizontal black line inside the motor head represents the region within the conserved motor domain covered by the 14 exons screened for mutations.

mutation was observed in one USH1B family with the mother and her two affected sons being heterozygous for the deletion.

A single base deletion in exon 5 between codons 120 and 121 (CGCCAG→CGCAG) causes a frameshift ending in an UGA stop after 24 amino acids. This mutation was observed in the heterozygous state in two affected siblings and their mother.

A single base deletion in exon 14 at codon 532 (CAC) occurred in one Usher 1b family. The mother and three affected sibs were all heterozygous for this deletion. The mutation causes a frameshift of 78 codons and ends at a UGA within the actin binding domain of the myosin VIIa molecule.

Two Usher 1 cases harbored separate nonsense mutations in exon 9. The first was a heterozygous G→T transversion in codon 314 (GAG→TAG) carried in an isolated USH1B case. The other nonsense mutation is a homozygous T→G transversion in codon 333 (fig. 4).

Polymorphisms

Several base substitution polymorphisms were detected by HA and sequencing in both affected and control sample groups (table 3). Weil et al. (1995) reported polymorphisms in exons 5 and 7. We have not determined whether the heteroduplex pattern observed in both sample groups for exon 5 is the same reported polymorphism. We did not observe any evidence of sequence variation in the control group for exon 7. The most-common polymorphism observed was in exon 3 and causes an amino acid change Ser16-Leu. It was found to have a heterozygosity approaching 50%. This can be typed by digestion of the exon 3 PCR product with *Sau*3AI. The 16Leu allele is refractory to digestion, while the 16Ser allele digests to 169-bp and 137-bp fragments.

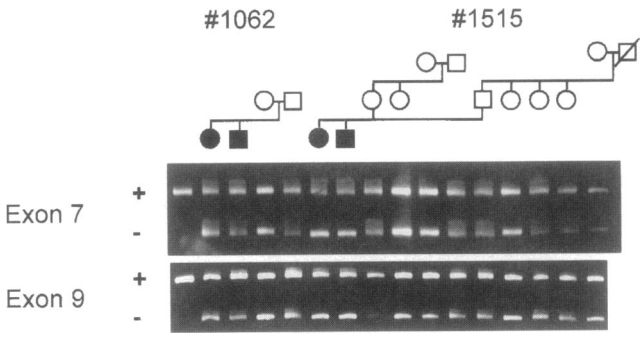

Figure 2 EtBr-stained MDE™ gel, showing heteroduplex patterns of exon 7 and 9 mutations. PCR products from families 1062 and 1515, which carry the Arg212His and Arg302His mutations. PCR products with (+) and without (−) cosmid 7D11 PCR control DNA were denatured and cooled slowly to allow the formation of heteroduplexes prior to gel electrophoresis. Exon 7 PCR products were loaded and run for 20 min at 1,200 V. Exon 7 products with the addition of 7D11 control were loaded in the same lanes and the gel was electrophoresed according to Subjects and Methods. Exon 9 PCR products with and without cosmid control PCR product were electrophoresed in a manner similar to that for exon 7. Family 1062 shows paternal inheritance of both Arg212His and Arg302His mutations with affected siblings being heterozygous. Affected sibs in family 1515 are homozygous for both mutations (note the absence of heteroduplex formation without the addition of 7D11 PCR product), with each parent being obligate heterozygotes.

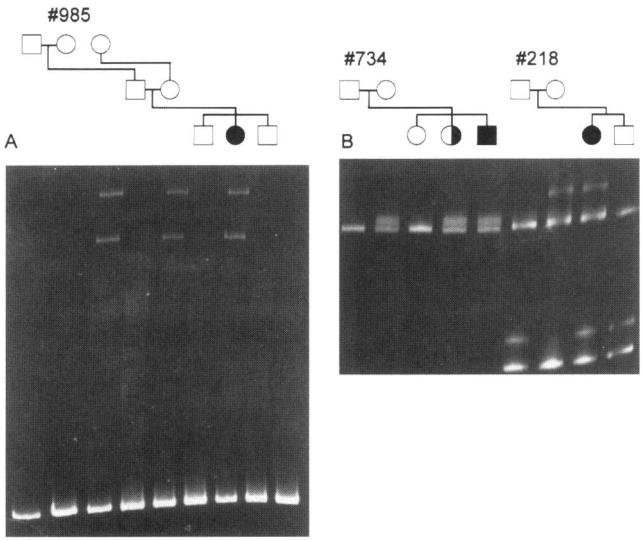

Figure 3 Segregation of exon 13 and exon 14 mutations in EtBr-stained MDE™ gels. *A*, Heteroduplex pattern of exon 13 PCR product (432 bp), showing the 468 + Gln mutation segregating in family 985. The proband, her mother, and maternal grandmother are heterozygous for a GCA insertion. The last lane on the right side of panel *A* is exon 13 PCR product amplified from 7D11. *B*, Heteroduplex patterns of exon 13 Pro503Leu (family 734), Glu450Gln, and the intronic IVS13nt-8c→g mutations (family 218). The proband from 218 carries two different mutations (maternal Glu410Gln, paternal IVS13nt-8c→g), while the unaffected brother is only heterozygous for IVS13nt-8c→g mutation. Family 734 is an unusual USH1B family. The sister is heterozygous for the Pro503Leu mutation and was diagnosed with a progressive hearing loss (half-filled symbol), probably unrelated to Usher syndrome.

Discussion

Myosins are a superfamily of molecular motors that interact with actin filaments, converting energy from ATP hydrolysis into mechanical force that is used to perform diverse cellular functions (Mooseker and Cheney 1995). Myosin VIIa is one of ⩾10 separate classes of unconventional myosins that are characterized as having a conserved N-terminal motor domain, a regulatory domain, and a unique tail domain. The recent characterization of the myosin VIIa tail reveals a short α-helical region predicted to dimerize myosin VIIa into a two-headed molecule (Hasson et al. 1995; Chen et al. 1996; Weil et al. 1996), and two repeated units each containing a novel MyTH4 domain similar to domains in three other myosins and a domain homologous to talin, a member of the band 4.1 family (Chen et al. 1996). Indirect immunofluorescence studies using antibodies specific for myosin VIIa protein have determined its location within tissues important to the pathology of Usher syndrome. Myosin VIIa was found to be limited to the inner and outer hair cells of the guinea pig organ of Corti and to the retinal pigmented epithelium (RPE) in adult rat (Hasson et al. 1995). Using a human-specific myosin VIIa antibody, El-Amraoui et al. (1996) recently demonstrated that myosin VIIa was localized in the human adult retina to the RPE cell layer, as well as the rod and cone photoreceptors cells, and confirmed that expression in rodents was limited to the RPE. However, it is not clear from these observations that the RP in USH1B is caused by myosin VIIa defects in the RPE, the rods and cones or both cell types. Indeed, the function of myosin VIIa may be different in the two affected sensory organs. If so, it is tempting to speculate that tissue-specific differences in function could lead to differences in the effect of specific mutations in the eye versus the inner ear. Alternative splicing is one mechanism for creating related proteins with varied functions. A number of alternate splice forms of MYO7A have been found, but the significance of these different forms is not known (Weil et al. 1996; Chen et al. 1996). Alternatively, myosin VIIa may have a functionally different, although biochemically similar, function in different types of cells. Myosin VIIa is highly expressed in Sertoli cells of the testis, an obvious nonsensory organ, but there has been no reported effect of Usher Ib mutations on spermatogenesis or fertility. The hypothesis of functional differences of myosin VIIa in the eye versus the inner ear is consistent with the observation that one type of recessive nonsyndromic hearing loss maps to the same region as MYO7A on 11q. Furthermore, the pattern of the disease in the eye versus the ear is different: the RP is partial and progressive with age, but the hearing loss is total and congenital. Consequently, we would predict that, as the nature and distribution of the MYO7A mutations are uncovered, the types of mutations and their distribution to specific functional domains occurring in the MYO7A gene will be correlated with specific differences in phenotype.

While the sample reported on here consists only of Usher I patients, mutation surveys of MYO7A in patients with isolated RP or isolated hearing loss are expected to be carried out. The effect of different mutations on the severity of the Usher I phenotype or their correlation with atypical cases of Usher I have not yet been investigated. While our experience suggests that there is little variation in the phenotype of Usher Ib, this impression is influenced by a severe bias in the criteria for the ascertainment of our families for linkage studies. If there were Usher Ib cases with milder hearing losses, normal vestibular responses, or other atypical features, they would not have been included in the original study. We are also in the process of organizing a mutation screening of atypical cases to compare with the results obtained for typical Usher Ib cases.

In the absence of a comprehensive biochemical picture of the role of myosin VIIa in cellular function, it is difficult to predict which mutations are pathologic and which are benign. This is especially problematic in a

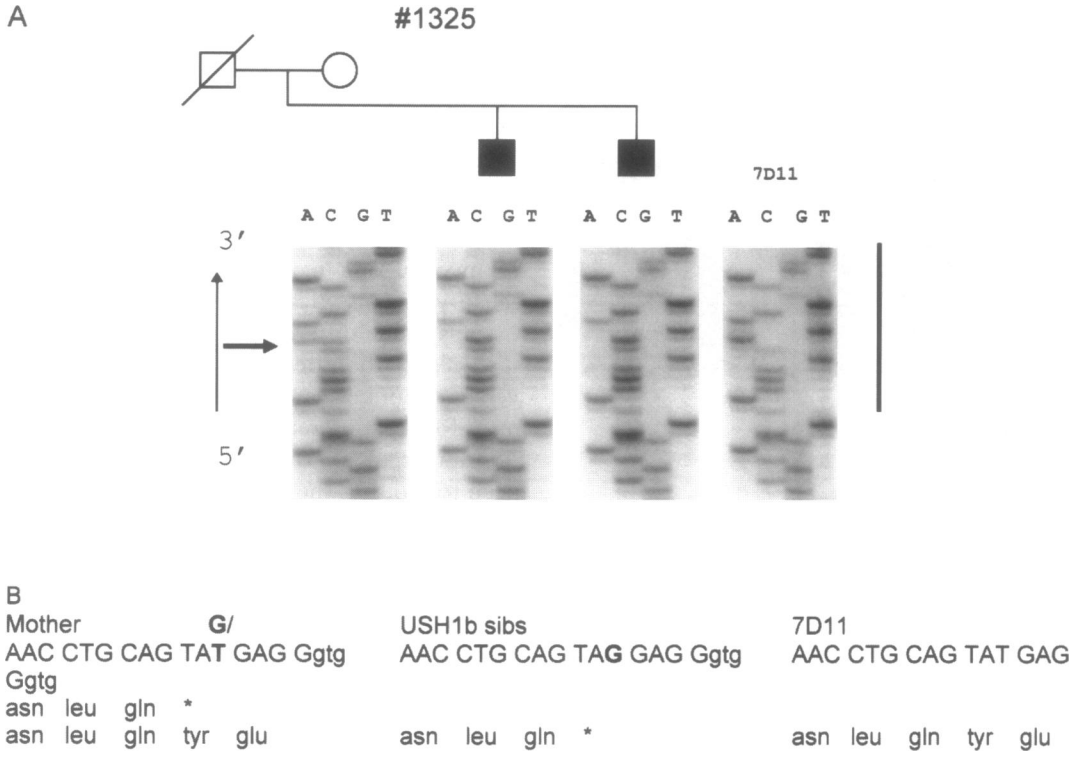

Figure 4 Tyr333Stop mutation in exon 9. *A*, Sequencing gel from family 1325. Exon 9 PCR product from family 1325 was sequenced using MYO7-9L primer (table 1). The sequence is, therefore, from the complementary strand. The 5′- to-3′ direction is shown. The arrow points to the heterozygous C/A of mother. The corresponding position in the affected siblings are homozygous C. *B*, Consequences of the base alteration to the amino acid sequence. The vertical black bar to the right of panel *A* corresponds to the region of the sequence whose complement is shown translated. The G→T transversion changes a tyrosine to a stop codon.

recessive disorder like Usher Ib, where only a few cases have been identified, to date, in which both mutant alleles are known. It is presumed here that all null mutations will cause disease in the homozygous state or when paired with a different null mutation. Alterations in the amino acid sequence of the myosin VIIa polypeptide are not always pathologic, and each mutation must be considered in light of the predicted disruption of function. This is a problem, since no one is completely sure of the true function of myosin VIIa in sensory cells. However, the availability of the three-dimensional structure of chicken skeletal myosin S1 (Rayment et al. 1993*b*) is an important resource for predicting the functional significance of particular domains of any myosin gene, since the primary structure of the N-terminal motor portion of the myosin superfamily has been highly conserved throughout evolution. There was 61% sequence similarity between myosin VIIa and chicken skeletal myosin at the amino acid level in the motor domain. A similar strategy for evaluating mutations was described for β-cardiac myosin II (MYH7) (Rayment et al. 1995), where missense mutations in this myosin gene have been shown to cause autosomal dominant familial hypertrophic cardiomyopathy (FHC). For this disease, the mutated protein appears to exert a dominant negative effect. Thirty-three of 40 MYH7 mutations occur in the motor domain, indicating that alterations in the motor activity are more likely to lead to a viable, hypertrophic phenotype than tail mutations (Vikstrom and Leinwand 1996). Of the USH1B mutations reported

Table 3

Polymorphisms Observed in the Myosin VIIa PCR Products

Exon-Specific PCR product	Polymorphic Change
3	Ser16Leu, TCG→TTG
4	IVS3nt-88c→t
4	IVS3nt-7c→t
8	IVS7nt-3c→t
8	Gly261Gly, GGT→GGC
10	IVS9nt-35c→g
10	IVS10nt+75t→c
12	IVS11nt-8t→c
12	IVS12nt+8g→a
14	IVS13nt-42t→c
14	Asn535Asn, AAC→AAT

here, only the ATP pocket mutations Arg212His or Cys correspond to those domains functionally important in causing FHC.

The Arg212His or Cys mutations in exon 7 lie C-terminal to the ATP binding loop of the myosin VIIa motor domain at the base of the ATP binding pocket. This region is conserved in all myosins from all classes. Mutations in MYH7 have been observed to occur in the same conserved motif in FHC patients. In particular, the MYH7 mutations, Asn232Ser and Phe244Leu (Rayment et al. 1995), lie within the same conserved motif as the Arg212* mutations. Because of the absolute evolutionary conservation of residues including codon 212 within this region of the ATP binding pocket, it is expected that the Arg212His or the Arg212Cys mutations would severely impair the function of the motor. This region of the motor is thought to undergo conformational changes during the actin-myosin power stroke, and so improper head movement and/or incomplete conversion of the energy from ATP hydrolysis are possible consequences of mutations within this conserved domain (Watkins et al. 1995).

The MYO7A Glu450Gln and +468Gln mutations in exon 13 are within a highly conserved α-helix domain of the myosin motor involved in actin binding. The +468Gln mutation occurs in a region of the α-helix adjacent to the domain that contains the Phe513Cys FHC mutation. Interpretation based on the three-dimensional (3-D) structure of chicken skeletal muscle suggested that the MYH7 Phe513Cys mutation may modify the nature and timing of conformational changes during the contractile cycle (Rayment et al. 1995). The position of MYO7A +468Gln with respect to the MYH7 Phe513Cys in the 3-D myosin structure suggests that +468Gln may cause a similar loss of motor function. The MYO7A Glu450Gln mutation occurs in the molecular cleft that splits the 50-kD fragment into the upper and lower domain. This cleft provides a communication pathway between the nucleotide- and actin-binding sites (Rayment et al. 1995). The Pro503Leu mutation occurs in a nonconserved region of the motor, although the Pro503 is conserved across several, but not all, myosin types (Mooseker and Cheney 1995). By comparison to the 3-D structure of chicken skeletal muscle myosin II, the equivalent residue is Pro529 and produces a prominent bulge within an α-helix that runs from Pro516 to Phe542, and this region has a potential stereospecific interaction with actin (Rayment et al. 1993*a*). Specifically, this region occurs on the outer edge of the molecule and is implicated in the two-step reaction of binding of myosin to actin (Taylor 1993). So, while it is possible that this is a phenotypically neutral mutation because of the lack of primary sequence conservation among the unconventional myosins in this domain, the observed contacts between actin and myosin in this region imply that this is a significant alteration. In addition, the original *sh-1* mouse mutation (Arg→Pro) occurs one codon upstream at the equivalent human position 502 (Gibson et al. 1995). The identification of two pathologic mutations in separate species within this region of the motor suggests that this region is critical for proper function. Furthermore, Pro503Leu was not observed in any of the 192 control chromosomes.

The MYO7A Arg302His in exon 9 is a problematic change in Usher Ib patients and illustrates the difficulty in trying to correlate phenotype/genotype associations. The Arg302His alteration is in a nonconserved region of the motor domain. Arginine at codon 302 has been observed in MYO7A sequences from human (U17180), mouse (201683A), pig (U34226), and bull frog (U14379) (Gibson et al. 1995; Hasson et al. 1995; Weil et al. 1995). Two of five different MYO7A cDNA clones isolated from three independent cDNA libraries were found to have a histidine at position 302 (Chen et al. 1996), suggesting that this mutation occurs in normal populations. However, all 192 control chromosomes were negative for the heteroduplex pattern observed in 5/378 USH1B chromosomes with Arg302His. Nevertheless, the Arg302His change may be neutral with respect to the USH1B phenotype. Alternatively, it may act with other mutations that cumulatively disable the motor. The fact that Arg302His was observed independently twice in *cis* with Arg212His suggests this possibility. Alternatively this observation could just as likely be due to linkage disequilibrium.

The IVS13nt-8c→g mutation is analogous to three human examples of identical −8-bp splice-acceptor mutations reported in the literature, (two cases of β thalassaemia, one case of adrenal hyperplasia) (Krawczak et al. 1992). Sebillon et al. (1995) analyzed a t→g mutation at a position 8 bp upstream of the 3′ splice site in intron 2 of the β-globin gene by an in vitro splicing assay. In vivo, this mutation was observed in *trans* with a frameshift mutation on the other allele and resulted in a mild β+ thalassemia phenotype, suggesting that normally spliced β-globin mRNA was produced at reduced levels. In vitro, this mutation decreased the splicing efficiency 2–3-fold relative to the wild type and is correlated with a reduction of spliceosome formation. Thus, we would predict that the analogous IVS13nt-8c→g mutation would seriously impair the proper construction of an effective MYO7A mRNA.

One case reported here was homozygous for both Arg212His and Arg302His (fig. 2). Because Arg212His and Arg302His occur in *cis,* it is plausible that this double mutation is important to the USH1B phenotype. Double mutations have been reported in cystic fibrosis, with similar difficulties in assessing whether the occurrence of these mutations in *cis* are additive or compensatory to the phenotype (Savov et al. 1995). Since the

entire coding region for MYO7A has yet to be scanned for additional mutations in these families, it is still too early to draw any concrete conclusions pertaining to the possible cooperation of these mutations in *cis* specific to the Usher Ib phenotype. In general, the USH1B missense mutations will contribute additional information to the general function of the myosin motor by pointing out critical domains that, when altered, create functionally inactive molecules.

One aspect of the MYO7A mutation search we wanted to address was the reason for the phenotypic differences between *sh-1* and Usher Ib. Three of the seven *Sh-1* mutations, Arg241Pro (*sh-1*^{6j}), Arg502Pro (*sh-1*), and del646-655 (*sh-1*^{816SB}), are known (Gibson et al. 1995). In the case of *sh-1*, the defective myosin VII protein causes a phenotypic effect only in the inner ear. It appears that similar missense mutations in humans produce both retinal and cochlear degeneration. The two mutations, mouse Arg502Pro and human Pro503Leu, occur in the same domain yet produce different phenotypic results with respect to the eye. This is consistent with the species-specific difference in myosin VIIa protein localization observed in the retina (El-Amraoui et al. 1996).

More quantitative clinical studies of patients and heterozygotes are needed. There appears to be no overt clinical differences between the cases categorized by the types of mutations observed (missense, nonsense). Nonsense mutations would produce no protein, and heterozygotes of null alleles have a decreased dosage of protein, which seems sufficient to produce a normal phenotype. A missense mutation, however, could produce an aberrant protein present in the cells of a heterozygote. In light of these considerations, one might expect that missense heterozygotes would have mild hearing and/or visual problems but that null heterozygotes would be asymptomatic. The case in family 734 typifies this possibility. Further investigation into the effects of the gene in the "normal carriers" may be helpful in elucidating the function of MYO7A. An exhaustive search for MYO7A mutations in Usher Ib patients will certainly contribute and augment the various biochemical and histological studies now underway to understand the contribution of MYO7A to cell function.

Three other mutations in MYO7A have been reported elsewhere (Weil et al. 1995). These mutations include two nonsense mutations caused by single base substitutions carried by two families that were heterozygous, (Arg150Stop and Gln234Stop) occurring in exons 5 and 7, respectively. In addition, a 6-base deletion in exon 7, corresponding to the deletions of codons 218–219, were found as heterozygotes in two separate families, with one family segregating both the Arg150Stop and del218-219. This report presents 3 new USH1B cases where mutations have been found in both alleles and raises the total number of unique mutations in MYO7A to 15.

The most-common Usher Ib mutations observed to date are Arg212Cys, Arg212His, and Arg302His. Together, these comprise ~50% of the total mutations observed, but this is <3% of the total Usher Ib chromosomes studied. Furthermore, we have not yet observed any linkage disequilibrium between Usher Ib and several adjacent polymorphic markers used in previous linkage studies (W. J. Kimberling; unpublished observations). This is consistent with the hypothesis that there are several independently occurring mutations and no common USH1B allele.

The length of the MYO7A cDNA is reported to be 7,409 bp or 7,465 bp (Weil et al. 1996; Chen et al., in press). We screened the first 14 of 49 exons, which cover 26% of the coding portion of MYO7A. The part of the gene screened includes ~3/4 of the N-terminal motor portion but none of the light chain binding or tail domains. Thirteen unique mutations were detected by HA in 23 separate USH1B chromosomes. Consequently, we expect to find the majority of MYO7A mutations in the tail. If true, this distribution would be different from that observed for MYH7 mutations, where most have been found in the motor head. It is possible, though less likely, that mutations occur in the intronic regions of the MYO7A gene and/or a common mutation that results in relatively large deletions of the MYO7A gene exists. In the latter case, many patients would be hemizygous and no variation would be seen with heteroduplex analysis.

The study of mutations in MYO7A and their relationship to the Usher I phenotype is just beginning. We expect that further investigations will reveal more about the structure and function of the myosin VII molecule, and continued progress will result in an enhanced understanding of the molecular pathology of this disorder.

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