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RESEARCH ARTICLE

Twelve Novel Myosin VIIA Mutations in 34 Patients With Usher Syndrome Type I: Confirmation of Genetic Heterogeneity

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Usher syndrome is a heterogeneous autosomal recessive trait and the most common cause of hereditary deaf-blindness. Usher syndrome type I (USH1) is characterised by profound congenital sensorineural hearing loss, vestibular dysfunction, and prepubertal onset of retinitis pigmentosa. Of the at least six different loci for USH1, USH1B maps on chromosome 11q13, and the *MYO7A* gene has been shown to be defective in USH1B. *MYO7A* encodes myosin VIIA, an unconventional myosin, and it consists of 48 coding exons. In this study, *MYO7A* was analysed in 34 unrelated Usher type I patients by single-strand conformation polymorphism analysis and direct sequencing. We identified a total of 12 novel and unique mutations, all single base changes. In addition, we found a previously reported nonsense mutation (C31X) on nine alleles of a total of six patients from Denmark. Hum Mutat 13:133–140, 1999.

KEY WORDS: **Usher syndrome type I; myosin VIIA gene; genetic heterogeneity; mutation screening**

INTRODUCTION

Usher syndrome, or more appropriately Usher syndromes, are autosomal recessively inherited and both clinically and genetically heterogeneous conditions. Clinical features consist of hearing loss and retinitis pigmentosa (RP), the latter leading to blindness in adulthood. Current classification of Usher syndrome encompasses the degree or progression of hearing loss, the onset of RP, and the presence of labyrinthine defect. Type I has a profound congenital hearing loss, vestibular a- or hyporeflexia, and prepubertal onset of RP. To date, six different USH1 loci (USH1A-F) have been genetically mapped. USH1B is on chromosome 11q13.5 and corresponds to the most common USH1 subtype. The myosin VIIA gene (*MYO7A*; MIM # 276903) was shown to be mutated in USH1B families.

Myosins are molecular motors that use the energy from ATP hydrolysis to generate force and movement along actin filaments. All myosins share a conserved head (motor) domain with characteristic ATP- and actin-binding regions and have distinctive C-terminal tail domains. Unconventional myosins are implicated in intracellular movements, primarily in nonmuscle cells. Myosin VIIA belongs to this latter group of myosins. The N-terminal head domain of myosin VIIA is followed by a 126-amino acid neck domain containing five repeat units (IQ motifs) serving as binding sites for members of calcium-binding proteins (light chain-binding domain) [Cheney et al., 1993; Weil et al., 1996]. The tail begins with a short (78-amino acid) coiled-coil region that is probably the site of homodimerization [Weil et al., 1997]. The tail region consists of two direct repeat containing elements, a proximal sequence motif of several tail homol-

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ogy regions, and a distal one with significant homology to the N-termini of talin and other members of the band-4.1 superfamily of actin-binding proteins [Chen et al., 1996; Weil et al., 1996]. It has been suggested that myosin VIIA is targeted to the plasma membrane by its talin-like motifs. In the photoreceptor cilium, trafficking of newly synthesised phototransductive membrane or maintaining the diffusion barrier between the inner and outer segments was suggested to be a major function of myosin VIIA [Liu et al., 1997b].

Human *MYO7A* consists of 49 exons spanning approximately 114 kb of genomic DNA and produces a 7.5-kb message. The longest open reading frame predicts a protein of 2,215 amino acids [Levy et al., 1997]. So far, 29 distinct *MYO7A* mutations, dispersed throughout the gene, have been reported in Usher type I patients [Weil et al., 1995; Weston et al., 1996; Adato et al., 1997; Levy et al., 1997; Liu et al., 1997a]. Some mutations have been found in multiple families, although no mutation hotspot has been seen. Importantly, it has been shown that the murine *shaker-1* phenotype, characterised by deafness and vestibular dysfunction, results from mutations in the gene encoding myosin VIIA mapped to the syntenic murine region [Gibson et al., 1995]. The present study reports the results of a screening of the entire *MYO7A* gene (except for the first noncoding exon) in 34 Usher type I patients.

MATERIALS AND METHODS

Extraction of genomic DNA from blood leukocytes was performed by standard methods. Polymerase chain reaction (PCR) was carried out using 100 ng of genomic DNA. Most of the oligonucleotide primers used here have been described by Levy et al. [1997] and, for exons 4, 6, 8, 12, and 13, by Weston et al. [1996]. Exons 5, 10, 18, 22, 33, 39, 46, and 49 were amplified using the primers listed below; exon 5 in two fragments, 5′-CTCCACCTCCCTCTTCATCA-3′ and 5′-GTCTCGGCTGTTGCGTTTCA-3′, 5′-CCAGAGCACATCCGCCAGTA-3′ and 5′-AGGGGAGGTGATTTTCTACA-3′; exon 10: 5′-CTGGGGAAGCATTTAGTCAC-3′ and 5′-′CACATAGCCCAGAGCATCAC-3′; exon 18: 5′-CCTCTCAGCCTCGGGGACAC-3′ and 5′-TCCTCCAGCCACACCACAGC-3′; exon 22: 5′-GATGCCCCCTTCCCTCAGTA-3′ and 5′-ATGCTGGCTGAGTGGGTCTA-3′; exon 33: 5′-TCTGTCCCTCTGTCCCTCTC-3′ and 5′-CGTTTCCCTCCCCTGCTGTG-3′; exon 39: 5′-CTCCCTCTATTCGGCACAAG-3′ and 5′-CCGTAACAGCAGGTGAGGTC-3′; exon 46: 5′-GAGTGGGCAGGGGTGGTGTG-3′ and 5′-TTATCAATGCCCTGTTCTCC-3′; and exon 49: 5′-GCTGTGCTATGGTCTGAG-3′ and 5′-AGATGCCTCACTGGAAGAACA-3′.

Final concentration of reagents in a 25-μl reaction volume was 10 pmol of each primer pair, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 M of each dNTP, and 0.5 U of Taq DNA polymerase (Gibco). PCR was performed on a Hybaid Omnigene cycler using different amplification protocols (Table 1).

Single-strand conformation polymorphism (SSCP) and heteroduplex (HD) analyses were performed essentially as described elsewhere [Bunge et al., 1996] using two different gel conditions routinely [6% acrylamide (cross-linking 2.6), and 10% glycerol in 1× TBE buffer; 8% acrylamide (cross-linking 2.6) in 1× TBE buffer without glycerol], whereas for some exons a third gel recipe [8% acrylamide (cross-linking 1.3), 10% sucrose in 0.5× TBE buffer] was used to optimise detection sensitivity. Samples were electrophoresed at 12–30 W for 4–16 hr at room temperature or at 4°C. Gels were silver stained. Samples showing bandshift in SSCP and/or HD were analysed by direct sequencing, using standard protocols [Bunge et al., 1996].

RESULTS

We examined the 48 coding exons of *MYO7A* by SSCP+HD analyses and direct sequencing in a total of 34 unrelated patients (19 from Germany and 15 from Denmark) affected by Usher syndrome type I. Amplification products included the donor and acceptor splice sites and at least 40 bp of flanking intronic sequences (the exceptions being exons 22 and 33, in which case the forward primer was located, respectively, only 18 and 28 bp apart from the exon/intron boundary).

Twelve novel and unique, and likely disease-causing, *MYO7A* mutations were identified on a total of 12 chromosomes; four patients were compound heterozygotes for a total of eight different mutations, whereas another four were heterozygous for a different subset of four mutations with a second mutation still missing. In addition, a previously reported nonsense mutation (C31X) [Weston et al., 1996] was detected in six Danish patients. Table 2 summarizes the mutations revealed in this study, and Figure 1 shows the relative location of each mutation along a linear representation of the protein.

Four novel nonsense mutations were found and

TABLE 1. PCR Conditions Used for *Myosin VIIA* Mutation Screening*

Exon no.	2	3	4	5A	5B	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
PCR protocol:	3	1	1	7	8	1	1	1	1	10	7	1	1	8	1	1	3	7	1	5	5	7	1	5	3
Fragment	380	279	262	408	298	404	356	204	335	461	229	258	378	317	259	272	305	308	255	223	411	269	339	331	278

Exon no.	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
PCR protocol:	2	9	4	6	1	3	6	8	6	1	1	3	6	7	1	1	1	2	1	1	7	3	1	7
Fragment length (bp):	243	323	275	318	357	347	400	357	284	391	320	273	350	247	304	272	275	230	230	290	274	220	273	336

Protocol 1	Protocol 2	Protocol 3	Protocol 4	Protocol 5	Protocol 6	Protocol 7	Protocol 8	Protocol 9	Protocol 10
94°C, 3′	94°C, 3′	94°C, 3′	94°C, 3′	94°C, 3′	94°C, 3′	95°C, 2′	95°C, 2′	95°C, 2′	95°C, 2′
5× 94°C, 20″	35× 94°C, 20″	35× 94°C, 1′	5× 94°C, 30″	35× 94°C, 20″	n× 94°C, 20″	8× 94°C, 20″	8× 94°C, 30″	10× 94°C, 30″	5× 94°C, 20″
60°C, 20″	57°C, 20″	55°C, 1′	60°C, 1′	60°C, 20″	Ann, 20″	68°C, 20″	68°C, 30″	68°C, 30″	68°C, 20″
72°C, 45″	72°C, 45″	72°C, 1′30	72°C, 1′30	72°C, 45″	72°C, 20″	72°C, 20″	72°C, 30″	72°C, 30″	72°C, 20″
30× 94°C, 20″			35× 94°C, 30″			5× 94°C, 20″	5× 94°C, 30″	5× 94°C, 30″	5× 94°C, 30″
55°C, 20″	72°C, 5′	72°C, 5′	55°C, 1′	72°C, 5′	72°C, 5′	60°C, 20″	60°C, 2′	62°C, 30″	60°C, 1′
72°C, 45″			72°C, 1′30		Ann n	72°C, 20″	72°C, 1′	72°C, 30″	72°C, 1′30
				+4% Formamide	65°C 5	30× 94°C, 30″	30× 94°C, 30″	25× 94°C, 30″	10× 94°C, 30″
72°C, 5′			72°C, 5′		60°C 15	54°C, 30″	54°C, 2′	57°C, 1′	57°C, 1′
					55°C 15	72°C, 30″	72°C, 30″	72°C, 30″	72°C, 1′30
						72°C, 5′	72°C, 5′	72°C, 5′	15× 94°C, 30″
									54°C, 1′
									72°C, 1′30
									72°C, 5′

*Nomenclature of exons according to Kelly et al., 1997.

TABLE 2. Likely Disease-Releated *Myosin VIIA* Mutations Detected in 34 USH1B Patients*

Mutation	Exon	Nucleotide change	Change in restriction map[a]	Genotype
Missense				
Arg241Ser	7	CGT→AGT	–*Afl*III	Compound heterozygote (/Tyr1719Cys)
Arg1240Glu	29	CGG→CAG		Heterozygote
Ala1288Pro	30	GCC→CCC		Compound heterozygote (/Arg666Stop)
Arg1343Ser	31	AGG→AGC		Heterozygote
Ala1628Ser	36	GCC→TCC		Heterozygote
Tyr1719Cys	37	TAT→TGT	+*Mae*III	Compound heterozygote
Gly2163Ser	48	GGC→AGC		Compound heterozygote (/IVS24-21g→a)
Nonsense				
Leu16Stop	3	TTC→TAG	+*Eco*NI	Compound heterozygote (/Gln1798Stop)
Cys31Stop[b]	3	TGC→TGA		Homozygote, heterozygote
Arg666Stop	17	CGA→TGA		Compound heterozygote
Glu960Stop	23	GAG→TAG		Heterozygote
Gln1798Stop	39	CAG→TAG	+*Mae*I	Compound heterozygote
Splicing				
IVS24-21	24	g→a		Compound heterozygote

*Codon numbering starts with the first in-frame methionine of myosin VIIA [Hasson et al., 1995; Weil et al., 1996; Chen et al., 1996].
[a]+, –, creation or elimination of a restriction site, respectively.
[b]Reported by Weston et al. (1996).

shown to cosegregate with the disease chromosome in the respective family (data not shown). Six apparently unrelated patients from Denmark carried the C→A transversion in exon 3 at codon 31 (C31X), already detected in a proband from Sweden and in a proband of Scandinavian ancestry from the USA [Weston et al., 1996]. Of the six Danish cases, three were homozygous, while the remaining three were heterozygous for the C31X mutation with a second mutation still missing. Although our inquiry has demonstrated no obvious anamnestic family relationship among these probands, genotyping for six intragenic polymorphisms suggests that the nine mutation bearing chromosomes originate from the same ancestor (data not shown).

Altogether, seven missense mutations were identified, all of which were observed only once in our patient sample and not found in 30 unaffected

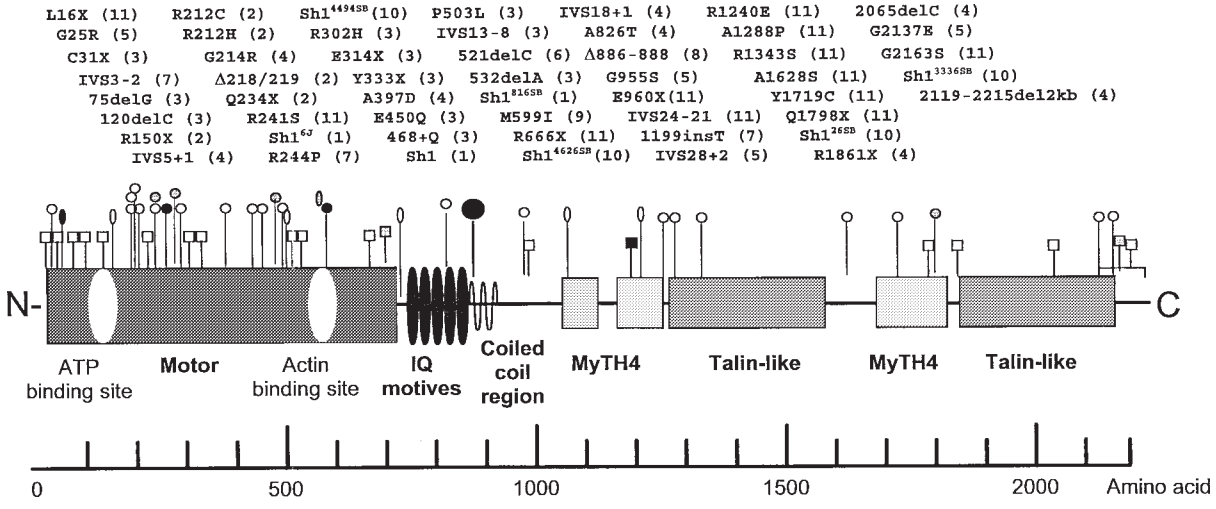

FIGURE 1. Schematic representation of the relative linear location of mutations along the myosin VIIA gene [compiled from Gibson et al., 1995 (1); Weil et al., 1995 (2); Weston et al., 1996 (3); Adato et al., 1997 (4); Levy et al., 1997 (5); Liu et al., 1997a (6), 1997c (7), and 1997d (8); Weil et al., 1997 (9); Mburu et al., 1997 (10), and this report (11)]. The gene structure is according to Chen et al. [1996] and Weil et al. [1996]. Domains of myosin VIIA are described in the text. *Circles*, missense mutations or small in-frame insertions or deletions; *squares*, nonsense mutations or frameshifts due to insertions or deletions; *ellipses*, splice-site mutations. *Open symbols*, the positions of the 41 mutations identified in patients with Usher type 1B; *gray symbols*, the seven mutations in the *shaker-1* mice; *black symbols*, the four mutations in DFNB2 families (small circles), and the 9-bp in-frame deletion in family DFNA11 (*larger black circle*) reported by Liu et al. [1997d].

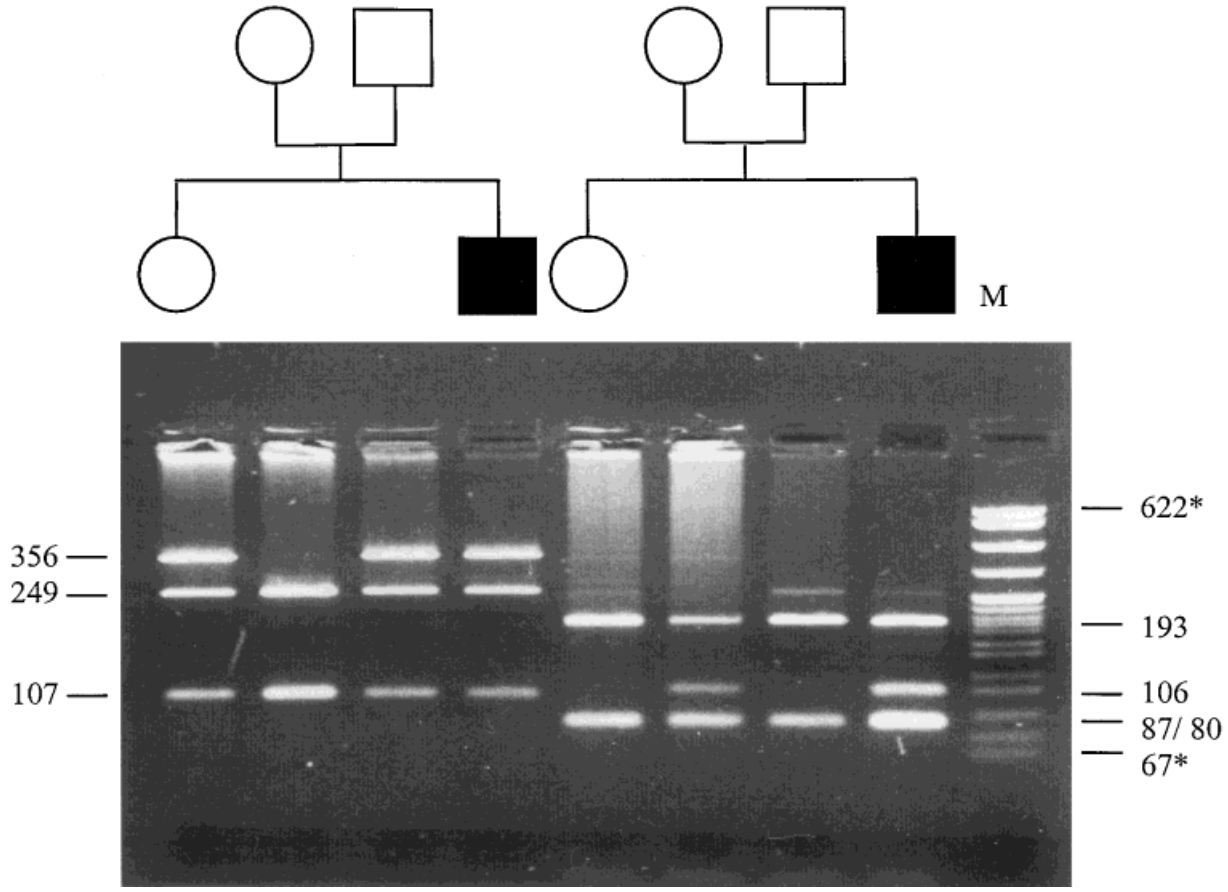

FIGURE 2. **Cosegregation of mutations at codon 241 and codon 1719 in Usher family #94. *Left*: Detection of the C→A transversion at position 721 in exon 7 by restriction digestion with AflIII. The mutation abolishes the recognition site at position 249 of the 356-bp amplicon. The father, his affected son, and his unaffected daughter all are heterozygous. *Right*: Detection of the A→G transition at position 5156 in exon 37 by restriction digestion with *Mae*III. The mutation introduces an additional recognition site at position 186 of the 273-bp amplicon. Both the mother and the affected son are heterozygous, whereas the unaffected daughter and the father do not carry the mutation. Numbers refer to the size of fragments in base pairs. M, molecular-weight marker, pBR322, *Msp*I digested. *, fragments of MW marker.**

controls (see also Fig. 2). In addition, two rare, and novel heterozygous variants, prediciting the exchange of residues 597 and 1992, and two silent third-position G→A transitions, in codons 761 and 1873, have also been observed (Table 3). Val-597Leu represents a conservative change of a residue that is evolutionarily conserved between the human and murine myosin VIIA, but not in different myosins in human. It was observed in a patient carrying the Gly2163Ser and the putative branchpoint mutation in intron 24 (IVS24-21g→a). The Phe1992Ile substitution in exon 44 was found *in cis* with the same sense variant Arg761Arg in exon 20 in a Danish family with two siblings of affected by Usher syndrome type II. Both sequence changes are on the maternal allele of the affected siblings, whereas they inherited different paternal *MYO7A* alleles as evidenced by genotyping for the common 22-bp deletion in intron 27. Taken together, we consider the four above-mentioned sequence variants, including the two amino acid replacements, most likely unrelated to USH1B.

The functional significance of the mutation IVS24-21g→a, which was absent in 30 controls and found in a patient carrying a heterozygous Gly2163Ser mutation, is unclear at this moment, it may affect correct splicing by altering the branchpoint sequence.

Several exonic and intronic base substitutions were detected both in affected and control samples and considered most likely disease-unrelated rare variants or polymorphisms (Table 3). It seems that the already reported deletion of 22 nucleotides in intron 27 [Kelley et al., 1997] is a common polymorphism in our collection as well.

TABLE 3. Summary of *Myosin VIIA* Sequence Variants and Polymorphisms Detected in This Study

Novel variants and polymorphisms	Frequency[a]	Exon-specific PCR product	Nucleotide change
Val597Ile	1	15	GTC→ATC
Phe1992Ile	1	44	TTC→ATC
Arg761Arg	1	20	AGG→AGA
Arg1873Arg	1	40	CGG→CGA
IVS4-189t/g	8	5	agt(t/g)ct
IVS5+54c/g	2	5	aa(c/g)tgt
IVS10+65t/c	1	10	ca(t/c)tgg
IVS22+47a/g, +49dela	68	22	cct(a/g)t(Δa)cc
IVS22+59g/a	68	22	ccc(g/a)agg
IVS29+9g/a	1	29	gttcgtgc(g/a)tg
IVS33+21a/t	32	33	gc(a/t)cc
IVS33+89 t/c	32	33	aa(t/c)gc
Leu16Ser	12	3	TTG→TCG
Ser1585Ser	10	35	AGT→AGC
Cys1666Ser	4	36	TGT→AGT
Lys1905Lys	38	41	AAG→AAA
Ile1954Leu	1	43	ATC→CTC
Ser2080Ser	1	46	TCC→TCT
Lys2106Lys	46	46	AAA→AAG
Asn2173Asn	1	48	AAC→AAT
IVS9-35c/g	1	10	cct(c/g)cc
IVS27+10del22	31	27	cg(Δgg...ct)ga
IVS42-7a/t	30	43	tg(a/t)ccccag
IVS48+25a/g	1	48	gcct(a/g)gtg

[a]Number refer to frequency of the variant given at the second place and were determined by the analysis of 68 alleles of apparently unrelated patients with Usher syndrome type I.

DISCUSSION

Mutation screening of human *MYO7A* on 68 alleles of apparently unrelated patients with Usher syndrome type I showed a total of 21 (30.9%) likely pathogenic alterations in the coding sequence and flanking intronic sequences of the gene. In 5 of the 34 families studied by us, linkage to USH1B on 11q13 was suggested or could not be excluded, and *MYO7A* mutations were found in all affected subjects from these families. Our results thus confirm previous observations that mutations in *MYO7A* are common in Usher syndrome type I [Weston et al., 1996; Adato et al., 1997; Levy et al., 1997]. In addition, our data provide further evidence that there is a wide range of genetic heterogeneity in USH1B. Given that the C31X mutation is the only disease-related mutation identified in this study that has already been reported previously [Weston et al., 1996], we conclude from our findings and from the observations of others that the majority of the *MYO7A* mutations arose as a single event, and that only a very small proportion of USH1B alleles can be predicted in certain populations [Adato et al., 1997].

The Arg241Ser change identified in this study is located in the head domain of myosin VIIA. Alignment of myosin motor domain sequences shows that Arg-241 is a highly conserved residue within a region of high evolutionarily conservation, it is present in all known myosins, including cytoplasmic, skeletal, and smooth muscle myosins. Arg-241 is thought to be crucial for maintaining the integrity and the three-dimensional structure of the nucleotide-binding pocket [Rayment et al., 1993; Cope et al., 1996; Liu et al., 1997c]. Interestingly, an arginine-to-proline change in the corresponding codon has been found in the murine *myo7a* in the *shaker* mouse *sh1*^{6J}, which is considered a model for nonsyndromic recessive deafness caused by the *MYO7A* defect [Gibson et al., 1995]. Both the human and the murine mutation may impair enzymatic activity of myosin VIIA by altering the ability of ATP binding. Moreover, it has recently been shown that the amount of the protein is decreased to 20% of the wild-type allele in *sh-1* mice despite normal myosin VIIA mRNA levels, suggesting that the mutant protein is largely unstable [Hasson et al., 1997].

Six of the seven different, likely pathogenic missense mutations described in this paper are in the tail region. Alanine-1288 is conserved between the human and murine myosin VIIA and is situated in a region with homology to human merlin and mouse talin, though this particular amino acid is not conserved therein. The same holds true for Arg1343Ser, Arg1240Glu, and Gly2163Ser, which are strictly conserved between the human and murine myosin VIIA, and are each in a conserved region with homology to the N-terminus of talin and

other members of the band-4.1 superfamily of actin-binding proteins [Chen et al., 1996; Weil et al., 1996]. In addition, the above-mentioned four mutated amino acids are evolutionarily conserved between human myosin VIIA and a 2,098-amino acid large polypeptide coded for by a *Caenorhabditis elegans* cDNA (GenBank accession # U80848) highly similar to myosin VIIA. Similarly, Tyr1917 is also conserved between the human and murine myosin VIIA and lies 20 amino acids upstream of the MyTH4 domain in a region of the protein that is strictly conserved between human and murine myosin VIIA, although it has no homology to other known myosins. The Ala1628Ser mutation occurred in a domain with significant homology to SH3 domains, whereas Ala-1628 itself was not found to be a conserved residue. SH3 is a small domain (~60 residues) found in a variety of signalling and cytoskeletal proteins, including the head of myosin II, what is thought to mediate the interactions regulating the activities of Ras-like small GTP-binding proteins.

Four novel nonsense mutations were identified and it is very likely that they all result in "functional null alleles," as shown for nonsense mutations in other cases. Mutations at branch sites have been shown to reduce the efficiency of splicing [reviewed in Padgett et al., 1986; Maslen et al., 1997]. Although the splicing branchpoint in higher eukaryotes is not well conserved, a preference for purines or pyrimidines at each position and the maintenance of the target A nucleotide can be observed. Examination of the last 60 nucleotides of intron 24 suggests that IVS24-25 to IVS24-19 is the likely branch site.

Each of the mutations Arg1240Glu, Arg 1343Ser, Ala1628Ser, and Glu960Stop was found in heterozygous state and, although we have repeatedly screened all *MYO7A* exons of these probands by SSCP and HD analyses using various experimental conditions, no second aberration was seen. Clearly, we may have missed the second mutation in the amplicons analysed, as neither SSCP nor HD, even if combined, detects all mutations present. In addition, certain types of heterozygous mutations, e.g., exon-spanning deletions or inversions, escape detection if single-exon amplification is used. We also cannot rule out the presence of disease-causing mutations in the promoter region or in introns.

In *MYO7A*, the total number of likely disease-related mutations identified so far is 46, including 23 missense mutations or in-frame deletions or insertions, 17 nonsense mutations or small insertions or deletions resulting in frameshift, and 6 splice-site mutations. Thirty of the mutations are localised in the head region, whereas this accumulation might be due to the higher screening effort concerning this region. Four of the *MYO7A* mutations have been reported in patients with autosomal recessive [Liu et al., 1997c; Weil et al., 1997], and a 9-bp deletion in exon 22 in patients with autosomal dominant nonsyndromic deafness [Liu et al., 1997d]. The latter mutation is the only known mutation situated in the coiled-coil domain of myosin VIIA, that has been suggested to be the site of homodimerization. Indeed, a defect of this functional feature would explain a dominant negative effect of this mutation.

The observations gathered to date seem to be in line with the hypothesis that many of the recessive mutations in *MYO7A* are pathogenic by loss of function. Yet, the precise mechanism by which mutations in human *MYO7A* lead to hearing loss and visual impairment at the same time is unknown. Analysis of the mutations found in patients with Usher syndrome type I, the *shaker-1* mice, and families with autosomal recessive (DFNB2) and dominant (DFNA11) nonsyndromic deafness suggests that the enzymatic activity of myosin VIIA is critical for its function in the inner ear. The phenotype of *shaker-1* mice, an animal model for autosomal recessive nonsyndromic deafness, but not for Usher syndrome, due to murine *myosin VIIA* mutations, could thus be explained by the observed reduction in protein level [Hasson et al., 1997]. The presence or absence of retinal degeneration remains unexplained. Different proteins might in some cases compensate for the lack of myosin VII in the photoreceptor cell. The identification of binding partners of the diverse myosin tail domains might elucidate the differences in phenotypes caused by different *myosin VIIA* alleles. Gene diagnosis is now feasible and could provide important insights for the genotype/phenotype correlation and is an important step toward a future treatment or prevention.

REFERENCES

Adato A, Weil D, Kalinski H, Pel-Or Y, Ayadi H, Petit C, Korostishevsky M, Bonne-Tamir B. 1997. 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 61:813–821.

Bunge S, Fuchs S, Gal A. 1996. Simple and nonisotopic methods to detect unknown gene mutations in nucleic acids. In: Adolph KW, editor. Methods in molecular genetics. Vol 8. Orlando, FL: Academic Press. p 26–39.

Chen Z-Y, Hasson T, Kelley PM, Schwender BJ, Schwartz MF, Ramakrishnan M, Kimberling WJ, Mooseker MS, Corey DP. 1996. Molecular cloning and domain structure of Human

myosin VII, the gene product defective in Usher syndrome 1B. Genomics 36:440–448.

Cheney R, Riley MA, Mooseker MS. 1993. Phylogenetic analysis of the myosin super family. Cell Motil Cytoskeleton 24:215–223.

Cope MJTV, Whisstock J, Rayment I, Kendrick-Jones J. 1996. Conservation within the myosin motor domain: implications for structure and function. Structure 4:969–987.

Gibson F, Walsh J, Mburu P, Varela A, Brown KA, Antonio M, Beisel K, Steel KP, Brown SDM. 1995. A type VII myosin encoded by the mouse deafness gene shaker-1. Nature 374:62–64.

Hasson T, Heintzelmann MB, Santos-Sacchi J, Corey DP, Mooseker MS. 1995. Expression in cochlea and retina of myosin VIIa, the gene product defective in Usher syndrome type 1B. Proc Natl Acad Sci USA 92:9815–9819.

Hasson T, Walsh J, Cable J, Mooseker MS, Brown SDM, Steel KP. 1997. Effects of shaker-1 mutations on myosinVIIa protein and mRNA expression. Cell Motil Cytoskeleton 37:127–138.

Kelley PM, Weston MD, Chen Z-Y, Orten DJ, Hasson T, Overbeck LD, Pinnt J, Talmadge CB, Ing P, Mooseker MS, Corey D, Sumegi J, Kimberling WJ. 1997. The genomic structure of the gene defective in Usher syndrome type Ib (MYO7A). Genomics 40:73–79.

Levy G, Levi-Acobas F, Blanchard S, Gerber S, Larget-Piet D, Chenal V, Liu X-Z, Newton V, Steel KP, Brown SDM, Munnich A, Kaplan J, Petit C, Weil D.. 1997. Myosin VIIA gene: heterogeneity of the mutations responsible for Usher syndrome type IB. Hum Mol Genet 6:111–116.

Liu X-Z, Newton VE, Steel KP, Brown SDM. 1997a. Identification of a new mutation of the myosin VII head region in Usher syndrome type I. Hum Mutat 10:168–170.

Liu X, Vansant G, Udovichenko IP, Wolfrum U, Williams DS. 1997b. Myosin VIIa, the product of the Usher 1B syndrome gene, is concentrated in the connecting cilia of photoreceptor cells. Cell Motil Cytoskeleton 37:240–252.

Liu X-Z, Walsh J, Mburu P, Kendrick-Jones J, Cope MJTV, Steel KP, Brown SDM. 1997c. Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. Nature Genet 16:188–190.

Liu X-Z, Walsh J, Tamagawa Y, Kitamura K, Nishizawa M, Steel KP, Brown SDM. 1997d. Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene. Nature Genet 17:268–269.

Maslen C, Babcock D, Raghunath M, Steinmann B. 1997. A rare branch-point mutation is associated with missplicing of fibrillin-2 in a large family with congenital contractural arachnodactyly. Am J Hum Genet 60:1389–1398.

Mburu P, Liu XZ, Walsh J, Saw D Jr, Cope MJTV, Gibson F, Kendrick-Jones J, Steel KP, Brown SDM. 1997. Mutation analysis of the mouse myosin VIIA deafness gene. Genes Funct 1:191–203.

Padgett RA, Grabowski RJ, Konarska MM, Seiler S, Sharp PA. 1986. Splicing of messenger RNA precursors. Annu Rev Biochem 55:1119–1150.

Rayment I, Rypniewski WR, Schmidt-Base K, Smith R, Tomchick DR, Benning MM, Winkelman DA, Wesenberg G, Holden HM. 1993. Three-dimensional structure of myosin subfragment-1: a molecular motor. Science 261:50–58.

Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, Mburu P, Varela A, Levilliers J, Weston MD, Kelley PM, Kimberling WJ, Wagenaar M, Levi-Acobas F, Larget-Piet D, Munnich A, Steel KP, Brown SDM, Petit C. 1995. Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature 374:60–61.

Weil D, Levy G, Sahly I, Levi-Acobas F, Blanchard S, El-Amraoui A, Crozet F, Philippe H, Abitbol M, Petit C. 1996. Human myosin VIIA responsible for the Usher 1B syndrome: a predicted membrane-associated motor protein expressed in developing sensory epithelia. Proc Natl Acad Sci USA 93:3232–3237.

Weil D, Kussel P, Blanchard S, Levy G, Levi-Acobas F, Drira M, Ayadi H, Petit C. 1997. The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. Nature Genet 16:191–193.

Weston MD, Kelley PM, Overbeck LD, Wagenaar M, Orten DJ, Hasson T, Chen Z-Y, Corey D, Mooseker M, Sumegi J, Cremers C, Moller C, Jacobson SG, Gorin MB, Kimberling WJ. 1996. Myosin VIIA mutation screening in 189 Usher syndrome type I patients. Am J Hum Genet 59:1074–1083.