Defective myosin VIIA gene responsible for Usher syndrome type 1B

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USHER syndrome represents the association of a hearing impairment with retinitis pigmentosa[1] and is the most frequent cause of deaf–blindness in humans. It is inherited as an autosomal recessive trait which is clinically and genetically heterogeneous[2,3]. Some patients show abnormal organization of microtubules in the axoneme of their photoreceptors cells (connecting cilium)[4–6], nasal ciliar cells[7] and sperm cells[5], as well as widespread degeneration of the organ of Corti[8]. Usher syndrome type 1 (USH1) is characterized by a profound congenital sensorineural hearing loss, constant vestibular dysfunction and prepubertal onset of retinitis pigmentosa. Of three different genes responsible for USH1[9–11], *USH1B* maps to 11q13.5 (ref. 10) and accounts for about 75% of USH1 patients[2,3]. The mouse deafness *shaker-1* (*sh1*) mutation has been localized to the homologous murine region[12,13]. Taking into account the cytoskeletal abnormalities in USH patients, the identification of a gene encoding an unconventional myosin as a candidate for *shaker-1* (ref. 14) led us to consider the human homologue as a good candidate for the gene that is defective in USH1B. Here we present evidence that a gene encoding myosin VIIA is responsible for USH1B. Two different premature stop codons, a six-base-pair deletion and two different missense mutations were detected in five unrelated families. In one of these families, the mutations were identified in both alleles. These mutations, which are located at the amino-terminal end of the motor domain of the protein, are likely to result in the absence of a functional protein. Thus USH1B appears as a primary cytoskeletal protein defect. These results implicate the genes encoding other unconventional myosins and their interacting proteins as candidates for other genetic forms of Usher syndrome.

A lambda subclone from YAC 965 (encompassing the *USH1B* locus[15]) was found to contain a 4.2-kilobase (kb) *Eco*RI fragment which hybridized to a 2-kb mouse genomic fragment comprising five exons of a myosin gene[14]. We identified ten exons by sequencing this fragment and the immediately adjacent 6-kb *Eco*RI fragment. Subsequent isolation of clones from a retinal complementary DNA library extended the sequence to 1,851 base pairs (bp) (NCBI accession number, U17180). The deduced amino-acid sequence encodes most of the motor head of myosin and is 96% identical to murine myosin. The human sequence includes a previously characterized sequence of 31 residues, referred to as myosin VIIA (ref. 16).

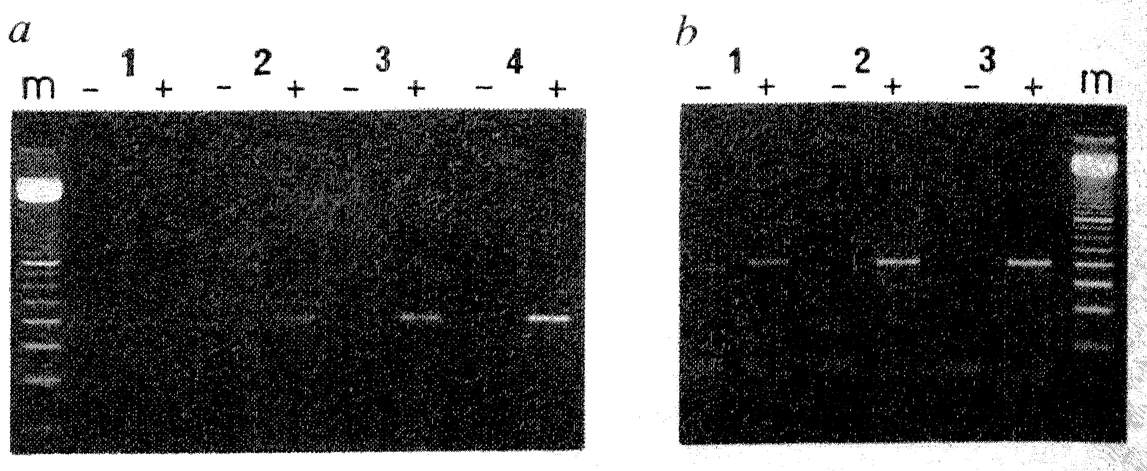

FIG. 1 RT-PCR analysis of myosin VIIA expression in human (*a*) and mouse (*b*) tissues. *a*, A 402-bp specific product was detected in liver (lane 2), kidney (lane 3) and retina (lane 4) but not in brain (lane 1). *b*, A 407-bp product is detected in retina (lane 1), cochlea (lane 2) and kidney (lane 3). The plus and minus signs indicate that the PCR reaction was done with or without reverse transcriptase. Lane m shows a 100-base ladder (BRL).
METHODS. Total RNA was transcribed using primer 1 (overlapping the junction between the fourth and fifth exons) and then amplified for 40 cycles with primer 1 and primer 2 (overlapping the first and second exons). Primer sequences were: human primer 1, 5′-TATGCAGTTACCCATGGCCAAG-3′; human 2, 5′-CTGCATCATCAGTGGGGAATC-3′; mouse primer 1, 5′-CAGGTTGATGCAGTTACCCATG-3′; mouse 2, 5′-CTGTATTATCAGCGGGGAAG-3′.

Expression of the myosin VIIA gene was studied using the reverse-transcription polymerase chain reaction (RT-PCR) technique. RT-PCR products of the expected sizes were detected in human kidney, liver and retina, but no RT-PCR product was seen in brain or in lymphocytes transformed by Epstein Barr virus (Fig. 1*a*); in the mouse, expression was detected in the retina, cochlea, kidney and liver (Fig. 1*b*). The sequences of the PCR products matched those of the isolated cDNAs.

A search for mutations was undertaken in nine unrelated families[3] in which segregation analysis indicated that the *USH1B* gene could be involved. The five 5′-most exons were amplified using intronic flanking primers and the PCR products sequenced. In three families, mutations were detected in four exons, confirmed by sequencing of independent PCR products subcloned in M13 vectors. In family 1, a heterozygous C→T transition in the first exon (nucleotide 163), resulting in a premature stop codon, was found in the two affected probands and their mother, but not in their unaffected brother and father (Fig. 2*a*). In family 2, a heterozygous C→T transition in the third exon (nucleotide 108), also resulting in a premature stop codon, was detected in the affected proband and his mother but not in his unaffected brother and father (Fig. 2*b*). In families 1 and 3, the same in-frame 6-base-pair (bp) deletion (GACATC) was observed in exon 3 (position 60 to 65), leading to loss of amino-acid residues D and I. In family 1, the two affected brothers and their father were heterozygous for the deletion. In family 3, the two affected probands and their mother, but not their father, carried the deleted allele (Fig. 2*b*). The finding of the same mutation in families originating from different geographical regions suggests that two independent mutational events have occurred, indicating a propensity of this site to mutation. Because this hexanucleotide deletion occurs in an 11-bp sequence containing two 5-bp direct repeats, it is tempting to speculate that either replication slippage or slipped-strand mispairing is responsible for the mutational event. In another series of ten families[2], two different heterozygous missense mutations in exon 3 were detected in two families at position 42 (C→T in family A) and position 43 (G→A in family B) (Fig. 2*b*). These mutations

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respectively substitute a cysteine and a histidine codon for the same arginine codon. None of these mutations was found in the 74 alleles from the general population that were sequenced as controls.

The identification of the mutations in both alleles of the affected children from family 1, and the cosegregation of each of these mutations with the disease, provide strong evidence that the myosin VIIA gene is responsible for USH1B. Moreover, the nonsense mutations found in exons 1 and 3 would result in proteins truncated before the ATP-binding site and the actin-binding site respectively and therefore not functional. The isoleucine present in the two-amino-acid deletion is a highly conserved residue in conventional (myosin II) as well as unconventional myosins (Fig. 3). These two residues are located inside a large β-sheet[17] and hence are likely to result in an incorrect folding of the motor domain and consequently in an inactive protein. The two substitutions of the arginine present in a highly conserved heptapeptide sequence (Fig. 3) close to the phosphate-binding site[17] would also be expected to impair the function of this myosin.

Our results and those described in the accompanying Letter establish that *USH1B* and *shaker-1* involve the orthologous myosin VIIA genes. But the phenotypes of the original *sh1* mutant and of the five other *sh1* mutants seem to be different from that of USH1 patients, because no sign of retinal degeneration has so far been reported in these mouse mutants[18,19]. Therefore either another myosin could compensate for a defective myosin VIIA in the mouse retina or the nature of the mutation itself[14] might account for the phenotypic difference. One form of human neurosensorial recessive deafness without retinal dystrophy, attributed to the gene *DFNB2*, which maps to the same chromosomal region as *USH1B*, may represent the human equivalent of the *sh-1* mutants[15].

Myosin I consensus[30]	:	FGNAKT.RNN**NSSRFGK**Y**m**E**I**q**F**D
Myosin II consensus[30]	:	FGNAKTvRNn**NSSRFGK**F**I**r**I**h**F**.
Myosin V mouse[31]	:	IGNAKTTRND**NSSRFGK**Y**I**E**I**GF**D**
Myosin VI human, pig[16]	:	FGNAKTVRNN**NSSRFGK**F**V**E**I**HF**N**
Human myosin VIIA	:	FGNAKTIRND**NSSRFGK**Y**I**D**I**HF**N**

FIG. 3 Amino-acid alignments in different myosins around the missense mutations and the 2-amino-acid deletion. The heptapeptide sequences containing the mutated arginine and the deleted isoleucine are indicated in bold.

Unconventional myosins are motor molecules with structurally conserved heads, which move along actin filaments using their actin-activated ATPase activity. Their highly divergent tails are presumed to be tethered to different macromolecular structures that move relative to actin filaments[20,21]. If we assume that the anomalous connecting cilium of photoreceptor cells described in some USH patients is associated with clinical subtype 1B, we have to postulate an interaction between actin filaments and microtubules involving myosin VIIA. The presence of myosin at the base of connecting cilia[22] is consistent with this postulate. According to this hypothesis, the kinocilium of cochlear and vestibular hair cells could be the primary defective structure in the inner ear[23]. However, such an interaction between microfilaments and the microtubule network is still poorly documented[24,25]. Alternatively, in the absence of direct evidence for abnormal microtubular structures in USH1B, a role for myosin VIIA is a possibility in the microvilli of the retinal pigment cells, the inner-ear supporting cells and in the hair-cell stereocilia. Such a role is consistent with the presence of unconventional myosins in intestinal cell microvilli[26] and at the tip of the hair-cell stereocilia, where one or several myosins are presumably responsible for modulating the tip-link tension that controls the gating of cation-transduction channels[27-29].

As there are at least seven genes responsible for Usher syndrome[2], it is possible that abnormalities in the genes encoding other unconventional myosins and their various binding components are responsible for other genetic forms of this dual sensory defect. □

a

```
1   ACGTATACGGGCTCCATCCTGGTGGCTGTGAACCCCTACCAGCTGCTCTC
      T  Y  T  G  S  I  L  V  A  V  N  P  Y  Q  L  L  S

51  CATCTACTCGCCAGAGCACATCCGCCAGTATACCAACAAGAAGATTGGGG
      I  Y  S  P  E  H  I  R  Q  Y  T  N  K  K  I  G  E

101 AGATGCCCCCCCCACATCTTTGCCATTGCTGACAACTGCTACTTCAACATG
      M  P  P  H  I  F  A  I  A  D  N  C  Y  F  N  M

                  T
151 AAACGCAACAGCCGAGACCAGTGCTGCATCATCAG
      K  R  N  S  R  D  Q  C  C  I  I  S
                  ↓
                 STOP
```

b

```
                                                    TA
1   CATTTGGGAATGCCAAGACCATCCGCAATGACAACTCAAGCCGTTTCGGA
      F  G  N  A  K  T  I  R  N  D  N  S  S  R  F  G
                                                 C  H

    ------>  ------>
51  AAGTACATCGACATCCACTTCAACAAGCGGGGCGCCATCGAGGGCGCGAA
      K  Y  I  D  I  H  F  N  K  R  G  A  I  E  G  A  K

          T
101 GATTGAGCAGTACCTGCTGGAAAAGTCACGTGTCTGTCGCCAGGCCCTGG
      I  E  Q  Y  L  L  E  K  S  R  V  C  R  Q  A  L  D
            ↓
           STOP

151 ATGAAAGGAACTACCACGTGTTC
      E  R  N  Y  H  V  F
```

FIG. 2 Nucleotide and deduced amino-acid sequences of the two myosin VIIA exons of the human gene containing the mutations. *a*, Exon 1; *b*, exon 3. The mutations are indicated above and below the normal sequences and the 6-bp deletion which was found in two different families is underlined. Arrows show the 5-bp direct repeat. A silent polymorphism was detected in exon 1 at the third position (G→A) and in exon 3 at position 83 (C→T). The primers used for sequencing were: 1a, 5′-ACTAACTCCGAGCCAGACC-3′; 1b, 5′-GTTACACAGCACAGAGTACAT-AG-3′; 3a, 5′-GATTGAGCAGCAGGTCTT-3′; 3b, 5′-CAATACGGGCAGCAAT-AC-3′.

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