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Genomic structure of the human unconventional myosin VI gene

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Abstract

Mutations in myosin VI (*Myo6*) cause deafness and vestibular dysfunction in Snell's waltzer mice. Mutations in two other unconventional myosins cause deafness in both humans and mice, making myosin VI an attractive candidate for human deafness. In this report, we refined the map position of human myosin VI (*MYO6*) by radiation hybrid mapping and characterized the genomic structure of myosin VI. Human myosin VI is composed of 32 coding exons, spanning a genomic region of approximately 70 kb. Exon 30, containing a putative CKII site, was found to be alternatively spliced and appears only in fetal and adult human brain. D6S280 and D6S284 flank the myosin VI gene and were used to screen hearing impaired sib pairs for concordance with the polymorphic markers. No disease-associated mutations were identified in twenty-five families screened for myosin VI mutations by SSCP analysis. Three coding single nucleotide polymorphisms (cSNPs) were identified in myosin VI that did not alter the amino acid sequence. Myosin VI mutations may be rare in the human deaf population or alternatively, may be found in a population not yet examined. The determination of the *MYO6* genomic structure will enable screening of individuals with non-syndromic deafness, Usher's syndrome, or retinopathies associated with human chromosome 6q for mutations in this unconventional myosin.

Keywords: Non-syndromic hearing loss; Deafness; Snell's waltzer

1. Introduction

Mutations in unconventional myosins have been identified in syndromic and non-syndromic deafness in humans and mice (reviewed in Friedman et al., 1999). Unconventional myosins comprise a group of 14 non-filament-forming myosins, defined by a conserved motor domain and different tail domains. Together with the class II group of conventional myosins that are expressed in muscle and non-muscle cells, they form a superfamily of motor proteins defined by their ability to bind actin and hydrolyse adenosine triphospate (ATP). Unconventional myosins have been implicated in many crucial cellular functions, including endocytosis, ion channel regulation, anchoring of stereocilia, and vesicle movement (Mermall et al., 1998).

An intragenic deletion in the unconventional myosin, myosin VI, was found to be the cause of deafness and abnormal vestibular function in Snell's waltzer mice (Avraham et al., 1995). Myosin VI differs from most other class-specific myosins in a number of features. The head domain contains a unique ~ 25 amino acid insertion at the position of a surface loop (Mooseker and Cheney, 1995). An evolutionary conserved threonine in the head domain is a potential phosphoryation site for a specific heavy chain kinase at amino acid residue 405 in humans and 406 in mouse (Avraham et al., 1997). Experiments using chicken myosin VI recently demonstrated a major phosphorylation site in the head domain of myosin VI and in vitro experiments suggest that p21-activated kinase may be responsible for this phosphorylation (Buss et al., 1998). Myosin VI also has a unique 53 amino acid converter domain in the neck region that enables this motor to move toward the pointed (−) end of actin filaments (Wells et al., 1999). All other myosins char-

Abbreviations: bp, base pair(s); CKII, creatine kinase II; cSNP, coding single nucleotide polymorphism; kb, kilobase(s); *MYO6*, Human myosin VI; *Myo6*, mouse myosin VI; NSHL, non-syndromic hearing loss; PAC, P1 artificial chromosome; RT-PCR, reverse transcription polymerase chain reaction; SSCP, single strand conformation polymorphism; *sv*, Snell's waltzer; UTR, untranslated region(s); YAC, yeast artificial chromosome

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acterized so far move toward the barbed (+) end of actin filaments. The neck region contains only a single IQ motif, the binding site for calmodulin molecules, whereas other myosins contain more than one (Mooseker and Cheney, 1995). The tail region consists of a coiled-coil domain, followed by a unique tail region that does not share homology to any other unconventional myosin tail. In the inner ear, myosin VI expression is concentrated in the cuticular plate and stereocilia rootlets of the inner and outer hair cells (Hasson et al., 1997).

Using fluorescent in situ hybridisation (FISH), human myosin VI was mapped to chromosome 6q13 (Avraham et al., 1997). Although estimates stand at over 120 genes for recessive deafness alone, only 60 non-syndromic hearing loss (NSHL) loci have been mapped to date, and none to 6q13 (Morton, 1991; Van Camp and Smith, 2000). Since mutations in both unconventional myosins VIIA and XV are associated with mouse and human deafness (Gibson et al., 1995; Liu et al., 1997a,b; Probst et al., 1998; Wang et al., 1998b; Weil et al., 1995), and mouse genes have been used successfully to identify genes involved in human deafness (Vahava et al., 1998), we hypothesized that myosin VI may be involved in human deafness as well.

2. Materials and methods

2.1. Refined mapping of MYO6

The G3 Radiation Hybrid panel (URL: http://shgc-www.stanford.edu/) was screened using polymerase chain reaction (PCR) primers designed from *MYO6* cDNA sequence (GenBank U90236) (H3504: 5′-CTGGTGGTA-TGCCCATTTTGA-3′ and H3789: 5′-CTACTTTAACAG-ACTCTGCAG-3′).

2.2. Identification of yeast artificial chromosomes (YACs) and P1 artificial chromosome (PACs)

YACs positive for D6S990 were obtained from CEPH Genethon (http://www.cephb.fr) and screened by toothpicking colonies as template for PCR using primers H3504 and H3789R for the 3′ region and primers E262 (5′-TTTCTAT-GAGCTTTGTTTATCTTT-3′) and H376R (5′-GAGG-TGGTCTTGTCCCAAGAG-3′) for the 5′ region. Myosin VI PACs P622P15, P696G4, and P749N6 were obtained by hybridisation using probes derived from the *MYO6* cDNA (Ioannou et al., 1994).

2.3. Identification of exon–intron junctions by subcloing from PACs

Three overlapping PACs were subcloned into pBluescript (pBS-KS$^+$) vectors (Stratagene, La Jolla, CA) using *Bam*HI and *Sac*I. Subclones were screened for *MYO6* inserts by hybridising with PCR-amplified portions of *MYO6* cDNA; the ends of positive clones were sequenced using T3 and T7 primers. Sequencing was performed using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit and an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

2.4. Identification of exon–intron junctions by long-distance PCR

Long-distance PCR uses two cDNA specific primers (designed at 200–300 bp intervals along the cDNA sequence) and Expand Long Template PCR System (Roche Molecular Biochemicals, Mannheim, Germany) to amplify large fragments of DNA containing introns (Uziel et al., 1996). PCR was done on both cDNA and genomic DNA, and when the genomic DNA templates yielded larger fragments, they were sequenced as described above using the cDNA primers used to initially amplify the fragments.

2.5. Identification of exon–intron junctions by the LKL–LKS linker adaptor method

The LKL–LKS linker adaptor method is a modification of vectorette PCR (Arnold and Hodgson, 1991; Riley et al., 1990). We constructed separate ligation mixtures of *Rsa*I-, *Alu*I- and *Hae*III-digested YAC and PAC clones with the LKL–LKS linker adaptor. LKL–LKS linker adaptors were formed by combining 2 nmoles each of LKL primer (5′-GCGGTGACCCGGGAGATCTGAATTC-3′) and LKS primer (5′-GAATTCAGATC-3′), 25 mM Tris-HCl PH 7.5 and H_2O to a total reaction of 100 μl, heating to 95°C for 5 min, followed by 65°C for 10 min and then decreasing 10°C each 10 min until 15°C. The ligation mixtures containing hybrids of genomic clone restriction fragments with the linker adaptor were made by combining 0.625 μg of restriction enzyme-digested genomic clone, 6.6 ul linker adaptor, 10× ligation buffer, rATP, 2.5 units of T4 DNA ligase (Stratagene, LaJolla, CA) and H_2O to a final volume of 50 μl and incubating overnight at 14°C. Amplification was performed with primers designed from known cDNA sequence (which determined the annealing temperature) and the LKL primer, using each ligation mixture (diluted 1:3) as a template to obtain PCR products containing exon–intron junctions. In general, an exon–intron PCR product was only obtained when the gene-specific primer contained contiguous exonic sequence that was not interrupted by an intron. The size of PCR products (200–1000 bp) varied among restriction enzyme depending upon the frequency and distribution of sites in genomic DNA.

2.6. Reverse transcription polymerase chain reaction (RT-PCR)

Human adult and fetal brain tissue RNA was obtained from Compugen Ltd. (Tel Aviv, Israel). The Human Fetal and Human I MTC Panels, which includes all tissues used in our study except for cochlea, was used as a source of cDNA (Clontech, Palo Alto, USA). Total cellular RNAs were

extracted (Chirgwin et al., 1979) from cochlea (membranous labyrinths) obtained from second trimester human fetuses, in accordance with guidelines established by the Human Research Committee at the Brigham and Women's Hospital. Three separate reverse transcription (RT) reactions were performed with Homo-Oligomeric DNA $d(T)_{12–18}$, Random Hexamer (Amersham Pharmacia Biotech, Piscataway, NJ), and *MYO6* primer E3789R (5′-GTTAAATCTGGGGGCACACAC-3′). A cDNA product was obtained from a mixture of the RT reactions using primers H3235 (5′-GAGAAGAATTTCATAGGAGAC-3′) and E3789R.

2.7. Linkage analysis

Genomic DNA was prepared from blood (Miller et al., 1988). Polymorphic markers D6S280 and D6S284, with a heterozygosity of 0.69 and 0.73, respectively, were genotyped. PCR products were labelled with P^{32}-dCTP incorporation during amplification, resolved on a 6% denaturing acrylamide gel and exposed to film.

2.8. Mutation analysis

Mutation detection was performed by single-strand conformation polymorphism (SSCP) (Orita et al., 1989) analysis, using primers we designed based on the exon–intron structure obtained. Samples were run on GeneGel Excel gels (Amersham Pharmacia Biotech, Piscataway, NJ) on a GenePhor electrophoresis unit at 45 W and 12 mA for 5.5 h at 7°C. Variant fragments obtained by SSCP were reamplified and sequenced as described above.

2.9. Coding single nucleotide polymorphisms (SNP) analysis

SNPs (Wang et al., 1998a) were identified as SSCP variants that did not change the predicted amino acid sequence. They did not cosegregate with deafness. Frequencies were determined from 56 chromosomes, represented by 25 deaf probands and three unaffected individuals. Variant fragments obtained by SSCP were reamplified and sequenced.

3. Results

We previously reported cDNA sequences of human and mouse myosin VI genes (GenBank Accession Numbers U90236 and U49739, respectively) and the chromosomal location of human myosin VI (*MYO6*) to 6q13 (Avraham et al., 1995, 1997). Screening the G3 Radiation Hybrid using PCR primers designed from *MYO6* cDNA sequence indicated that *MYO6* is approximately 21 cR (~600–700 kb) from WI-548 (D6S990), with a LOD score of 9.66.

A combination of three methods was used to identify *MYO6* exon–intron junctions. Nine exon–intron junctions were identified by sequencing subclones of PACs P622P15, P696G4, and P749N6. Ten exon–intron junctions were obtained by long-distance PCR. The LKL–LKS linker adaptor technique was the most efficient method of identifying exon–intron junctions, as it yielded 45 junctions. This technique worked well on both PACs and YACs.

Human myosin VI is composed of 32 coding exons (Table 1, Fig. 1), spanning a genomic region of approximately 70 kb. Exons 1–20 encode the head domain, including the ATP-binding domain (exon 5), the threonine 405 residue (exon 11) and the actin-binding domain (exon 19). The neck domain is encoded by exons 21–23, with the unique 53 amino acid converter domain encoded by exons 21–23 and the IQ motif by exon 23. The coiled-coil domain of the tail region is encoded by exons 24–27, and the remainder of the tail (the globular region) is encoded by exons 28–32. The exons range from 27–219 bp in size. The intron sizes, obtained by sequencing across introns or estimated by long-distance PCR, ranged from 0.04 to 5.6 kb. The genomic length was estimated by adding up the exon lengths, obtained by sequencing, and the intron estimated lengths. The consensus dinucleotides GT and AG were found at the donor and acceptor splice sites of all introns.

Exon 30 was found to be alternatively spliced. When sequencing cDNA derived from different tissues in which *MYO6* is known to be expressed (Avraham et al., 1997), two isoforms were detected only in human fetal and adult brain. Most human tissues examined, including fetal and adult heart, kidney, liver, skeletal muscle; adult cerebellum, colon, lymphocytes, placenta; and fetal cochlea, lung, spleen and thymus, were missing exon 30. Both fetal and adult brain contained two isoforms, one including the 27-bp exon 30, and the other excluding it. This differential splicing leads to the presence or absence of a putative casein kinase II (CKII) phosphorylation site, one out of 13 putative CKII sites identified in myosin VI using PROSITE (http://www.expasy.ch/prosite/). CKII is a protein kinase responsible for phosphorylation of Ser and Thr residues in a wide range of proteins, including cytoskeletal proteins, growth factor receptors, and transcription factors (Pinna, 1997). These sites contain acidic residues immediately downstream from the target Ser or Thr residues, with the most important one being $n + 3$ from the phosphorylation site. CKII has been shown to phosphorylate sites in vertebrate myosin II, where alternate splicing includes or excludes one of these sites (Kelley and Adelstein, 1994; Murakami et al., 1998). In the isoform excluding exon 30, the crucial $n + 3$ acidic amino acid is changed from an Asp to Ala, followed by a deletion of nine amino acids.

To determine whether *MYO6* mutations contribute to deafness in humans, we first screened hearing impaired sib pairs for concordance with *MYO6* flanking markers. Our refined map position of *MYO6* indicated that the polymorphic markers D6S280 and D6S284, with a heterozygosity of 0.69 and 0.73, respectively (http://www.gdb.org/), flank *MYO6* and were therefore chosen for performing linkage analysis. Segregation analysis was undertaken in 28

Table 1
Exon–Intron organization of the human myosin VI gene

Exon	5′ Intronic sequence	Exon first base[a]	5′ Sequence	Exon length (bp)	3′ Sequence	Exon last base	3′ Intronic sequence	Intron size 3′ (kb)
1[b]	aggtgacagtggatagtggaaacaggag	−21	ATCGTG	139	GGCAAG	117	gtgagtttctcagaaagatgttgaaatatg	4.9
2	gtttaatgagcatttgttttgcttgttag	118	ACATTT	70	ATAACT	187	gtaagtaccaagttaaaaattaactctccg	5.6
3	ctaatgacatataaattttatgtcttag	188	GTTCAC	74	ATTTAT	261	gtaagtattttacctgtagtgtaagtttttgt	1.7
4	atattttatgtttatgcttttcgtatttag	262	ACATAT	130	CAATTG	391	gtaagtgattttaaatgtatttttaattcttgtc	2.1
5	catcattaatgtcttattttgaccctag	392	CTGATA	106	TCTAAG	497	gtgagtattcagctaacttgaagtatttga	3.0
6	caacatttttttatcctatatttttaaacag	498	ATACCT	56	TTGAAG	553	gtattgctaatttttcagttgttacgcgtgta	4.6
7	tttttgatggatttttttgtttctcaatag	554	CTAACC	98	GAAAAG	651	gtaagtgagagtaagctttggaatgatatt	0.6
8	aaatatattttctctgtgttttgtttttttag	652	AGCTCA	165	TTTCGG	816	gtaggtcagaagaaaagaaatttcatata	3.4
9	gttaattatatttaatttgttttgtcatag	817	TATTTA	81	CCTGAG	897	gtatagtagaccattgttcataaaatcttta	0.6
10	cagttaattggtgtcttcttgttttgtag	898	TACCTT	181	CTTCAG	1078	gtttgctttttatttttttaagaggaaaaaaa	2.3
11	taatttcaatgaaaatatatttctattag	1079	GTGGTT	145	TATAAA	1223	gtaagttccttaagtaattgcactgcaaaa	2.1
12	aatttttactagcatagttttctttttatag	1224	GGTACC	158	GTTTTG	1381	gtaagtaaagtttctttttgtgagtatatattt	1.7
13	gactcttggttctggttttatattttcag	1382	AGTACT	92	AAGGAG	1473	gtaattgccattataagtttaatttaagatct	3.7
14	tatctatgattatgattatttcatttttag	1474	GAACAA	73	GTATAG	1546	gtatgtgtttttttaactccacctttgaaaaat	1.5
15	attgtgagtgttttcatttttttgaaatag	1547	ATTTAA	128	CTCACT	1674	gtgagttttgccattctgaaattgagactat	0.6
16	aataactcatatatgtattgttttttcag	1675	ATTCCC	96	GAAACA	1770	gtgagtataacttttttacaaggagaaaaa	0.6
17	gaaacattcctgtttgatttatttttcag	1771	ACCCAG	174	TTTAAG	1944	gtatttgtgttatttaatttttttttttactatattt	3.0
18	taataaactgatttccttttctttcacag	1945	ACACAG	39	AGTACT	1983	gtgagtatgcttaaaaagaaaacaggtttt	1.5
19	taacaaaaagtacctttatttttcctag	1984	GGAGCA	94	GTTCAG	2077	gtattttcataatctctgtcagtgtgtgttag	4.1
20	gtgctttgtgcttgttcgtgaatctag	2078	GGATGG	131	TGTAAG	2208	gtataaatgccacccaaattgaaatttctt	0.04
21	acatttaacttttttaacttttattttttcag	2209	GCTTTG	78	GGCAAG	2286	gtaaatatacatttttttactttaaactgtaac	1.7
22	ctgatactaaattatttcacttcctag	2287	TTTGCA	130	TCAAAT	2416	gtaggtgttttccttttacacctataggatctt	4.2
23	ctatcattttattttactcttacacatag	2417	TGAAAA	91	ACCTCG	2507	gtaagatgaatagttcctaaaaagaactc	0.8
24	aagctctaatgaagtattatttttacag	2508	CATTGA	151	ATTAAG	2658	gtatgtaatttcaacctgaatgactttgact	3.1
25	aaggaacctatttattttattttttaaag	2659	TCCCAT	209	GCGGAT	2867	gtgaggcatttatattattttgaataagaga	1.1
26	atttttgaagagttttctatttttttattag	2868	GAAACT	79	ATTCAA	2946	gtatgtacttactgggttgaatttctattaaa	0.4
27	ggtgaaatagctttttgcccttgtcag	2947	GTTGAA	161	GCGGAG	3107	gtactggggcccctgggtggggtatagc	1.7
28	catgcaaaaatgtgtaataattacag	3108	AGGTCC	104	CTTGTG	3211	gtaagtgtttggagaagatcaaaaataga	0.9
29	ttttttttttttgctcaatgtaatcaatag	3212	ATATTG	132	ATTATG	3343	gtaaagagaaatctgtactttttgaacgtttt	0.6
30	ttcttcaacataaaatatttgttcacag	3344	ATTTTG	27	ATTCAC	3370	gtaagtcaatgggtggtaactcatgagct	2.2
31	tataactttcccttgttctgtgtaatag	3371	CTCAGC	219	TGGCTG	3589	gtgtgtatgattcacatggaaaacaaatta	0.8
32	attttcctgatatcctcatgaatacag	3590	GTAAGG	200 or more				

[a] The first nucleotide of the open reading frame (translation initiation site) was designated as +1.
[b] The exon containing the first nucleotide of the open reading frame was designated as exon 1.

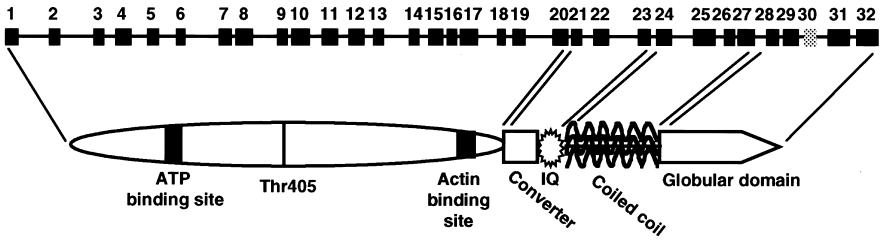

Fig. 1. Schematic diagram of the genomic structure of the human myosin VI gene. Coding exons are represented by black boxes; introns by lines separating each exon. Gene regions coding the head, neck and tail domains of the myosin VI protein are indicated. Exons and introns are drawn at different scales. The alternatively spliced exon is shown as a grey box.

families from the Middle East (Israeli and Palestinian), the UK, and the United States, with 2–13 hearing impaired members per family. Their hearing loss ranged from moderate to profound, with both recessive and dominant inheritance patterns, and prelingual and postlingual onset of hearing loss. Such a range was chosen since it has previously been shown that mutations in the same gene (myosin VIIA, connexin 26, and α-tectorin) can lead to hearing loss with variability in age of onset, mode of inheritance, and/or severity of deafness (Denoyelle et al., 1998; Kelsell et al., 1997; Liu et al., 1997a,b; Verhoeven et al., 1998). All families were excluded for mutations in the connexin 26 gene (Sobe et al., 2000). Linkage to D6S280 and D6S284 could not be excluded from 22 of these

Table 2
Primers used for SSCP mutation analysis of the myosin VI gene

Exon	Forward primer	Reverse primer	Product size (bp)
1	TTGAAACAGGTGACAGTGGAT	AGACAGACCTTACTCTTGTGA	254
2	AGCCCTTCTATTGTTCTTACTA	CAGTCTCCTTTTCTGTGCGG	181
3	TCAGATTAAGATAGTATTGCTTTA	CAAAATCAAAGAGTACCACATG	216
4	TTTCTATGAGCTTTGTTTATCTTT	CAATATAAAGAAAGACAAGAATTAA	257
5	GAGTAAGTGGTCCTAAAGTGA	ACAGTAAGACCCTATCTCAAG	265
6	GTACACCATATTATTGTCATCAA	AGAACATTCCAGACCACTAAAA	181
7	GAGATATACCATGCATATTTTGT	CAGCACCAACTGACCCTTG	241
8	GAAAAGGTAAATAATTTAACATTGG	CTGCACCTGGCTATATGAAATT	289
9	GGTAAGTTGTGTTTTCTCCTG	GTTAGAACTCTTACTTGGGCT	259
10	GACCTGGTGTAGTAGTTTTAG	GAGACAATTTTCATATGACAAATA	316
11	GTATAATTCTAAGAAATGGGTCT	GTAACTTATGTTTTTCCCTTGAC	296
12	GCACTCTGTGGCATTTTCAC	TAATGTGTGAAAAAATATGAAACAA	279
13	ACAATTACATTTTATCCTATGATAA	GTAACTGCAGCATATCTCTTC	250
14	ATGTTATGTTCAGAAACAGTGC	CACAGAAAAAGCACTAAACACA	223
15	TTAGTGAATTGTTCACATATCTTT	TAATATCTAATAGCTAATGCACC	279
16	CATCTCAGTTGTTTTGTAAAGAA	GACTAGAGCTATCATACTGTAC	246
17	CCTTTGGACAGAGCCATGTT	GATTTACATTATCCATTTTAGAATAT	318
18	ATGACACTGTGTACTTTGGCT	GCCTACTTGCATAATTACTAATA	168
19	GTTGCAGGTATTCATAGAAACA	GAAAATACTGAACCTATTAAATTG	241
20	TCTAAACAGTATCTTACAAAAATG	AAAAGTTAAATGTCAATTATTAATAG	298
21	ATTGAAATTTCTTAGCTATGAACT	CTTTAAGATATTCACTAACCCC	225
22	TTACATCATGTCTTTATGTGTTC	CCTTTACAACACTCCACAAAC	276
23	AAATGAGTTTTTTTAATATATGTTAG	TTATTATGACTTTATTGTCATTTTG	241
24	TCACTAAAGGATGAAATTAAGC	TATACAATTTCAATACCAATAACAA	274
25.1[a]	GTTAAGTATATTCCACATTGTTG	GTTTTTCGTCTTCTTCACGCT	253
25.2[a]	AGGAATTTCTCAGTGCATTACA	ATTAACCACTGTCAATACCAAG	220
26	AATCTGTTACCTTTGTTTATTATTA	ACCCAGCCTGTAGGTTCCAT	227
27	GTAATAAGGGGAGTGATCAAG	GGAAATGAGAGGCAGAGAGG	298
28	CAATGTTTTCTTCATGTTTCTGG	ACACCTCACCCTAATCTAGG	237
29	GACGAATAAATTAACATGTCAAT	TGTCTTAAGTACAGATACATGC	255
30	GCTCATTTAACAGTTTTGGCAT	CACTGAAAATTGTAGCAAAACA	188
31.1[a]	AAAGGCTCTTTTCTTTTCCCC	GGAGTTCCATTTGCCGGGC	256
31.2[a]	CTGCCGACCAGTACAAAGAC	CATTAGAATGTTTCATTCAGAGA	198
32.1[a]	CAGGCATACAACTGGTAAGAA	TGTCTGCTCTCAATCGCATTC	222
32.2[a]	TGGTGCTGAGATCTTGCCAAG	GTTAAATCTGGGGGCACACAC	210

[a] Two primer pairs were used for exons 25, 31, and 32 due to their length.

families. The entire coding region of *MYO6* was screened for mutations in these 22 families and in three additional families with only one hearing impaired child. No disease-associated mutations were discovered by SSCP analysis (Table 2) in all *MYO6* exons and exon–intron junctions. In the course of the sequence analysis, three cSNPs (Wang et al., 1998a) were detected which did not alter the amino acid sequence. They are as follows: exon 10, 1029C → T, with a frequency of 0.02 (determined from 56 chromosomes); exon 16, 1722C → T, frequency of 0.05; and exon 27, 2982G → A, frequency of 0.02.

4. Discussion

Loss of myosin VI in Snell's waltzer mice leads to deafness and vestibular dysfunction. A study on the development of sensory hair cells in these mice suggests that this unconventional myosin is involved in anchoring the apical hair cell membrane to the underlying actin-rich cuticular plate (Self et al., 1999). The identification of mutations in both human and mouse unconventional myosin VIIA and XV and the evolutionary conservation of myosin VI led us to speculate that some forms of human deafness may be caused by myosin VI mutations. We searched the entire *MYO6* coding region, including exon–intron boundaries, in DNA derived from 25 families with NSHL. No disease-associated mutations were identified in our study. We cannot, however, exclude the possibility of a mutation affecting the *MYO6* promoter or regulatory elements, which would affect expression levels. One Snell's waltzer allele, se^{sv}, results from an inversion affecting the upstream regulatory region of the *Myo6* gene (Avraham et al., 1995). In addition, ethnic and geographical boundaries may exist for specific mutations; for example, DFNB3 has been found so far only in India, Pakistan and Bali (Wang et al., 1998b). *MYO6*-associated deafness may exist in populations that have not yet been investigated. Alternatively, disease-associated mutations in *MYO6* could be very rare, explaining the fact that of all the NSHL loci identified to date in families around the world, none have yet been mapped near the *MYO6* region. Mutations in the *DIAPH1* and *POU4F3* genes have only been identified so far in one extended family each (Lynch et al., 1997; Vahava et al., 1998), and it may be that *MYO6* mutations are rare as well. Although *Myo6* mutations in the mouse lead to deafness (Avraham et al., 1995), species-specific expression and function may render mutations in human *MYO6* lethal or have a profound effect, leading to reduced viability. Such a case has been demonstrated for connexin 26, where null mutations in humans lead to deafness, but to lethality during mouse embryogenesis (Gabriel et al., 1998; Kelsell et al., 1997).

Furthermore, it may well be that mutations in human *MYO6* lead to a different phenotype than that observed in Snell's waltzer mice. Myosin VIIA mutations are the cause of deafness in shaker1 mice (Gibson et al., 1995). Most of the mutations in *MYO7A* in humans cause Usher syndrome 1B, which is characterized by profound congenital sensorineural deafness, vestibular dysfunction and prepubertal onset of retinis pigmentosa (Weil et al., 1995). Myosin VI may be associated with one of the unmapped Usher syndrome loci (Keats and Corey, 1999) or with another form of syndromic deafness, affecting the inner ear and other organs in which myosin VI is expressed. The retinopathies autosomal dominant Stargardt-like disease (ADSTGD), progressive bifocal chorioretinal atrophy (PBCRA), and North Carolina macular dystrophy (MCDR1) have been localized to the proximal long arm of chromosome 6 by linkage analysis (Gehrig et al., 1998). As this region contains the *MYO6* gene, it is possible that mutations in this unconventional myosin may cause one or more of these retinopathies in humans.

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