

# Mutation Profile of All 49 Exons of the Human Myosin VIIA Gene, and Haplotype Analysis, in Usher 1B Families from Diverse Origins

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## Summary

Usher syndrome types I (USH1A–USH1E) are a group of autosomal recessive diseases characterized by profound congenital hearing loss, vestibular areflexia, and progressive visual loss due to retinitis pigmentosa. The human myosin VIIA gene, located on 11q14, has been shown to be responsible for Usher syndrome type 1B (USH1B). Haplotypes were constructed in 28 USH1 families by use of the following polymorphic markers spanning the USH1B locus: D11S787, D11S527, D11S1789, D11S906, D11S4186, and OMP. Affected individuals and members of their families from 12 different ethnic origins were screened for the presence of mutations in all 49 exons of the myosin VIIA gene. In 15 families myosin VIIA mutations were detected, verifying their classification as USH1B. All these mutations are novel, including three missense mutations, one premature stop codon, two splicing mutations, one frameshift, and one deletion of >2 kb comprising exons 47 and 48, a part of exon 49, and the introns between them. Three mutations were shared by more than one family, consistent with haplotype similarities. Altogether, 16 USH1B haplotypes were observed in the 15 families; most haplotypes were population specific. Several exonic and intronic polymorphisms were also detected. None of the 20 known USH1B mutations reported so far in other world populations were identified in our families.

## Introduction

The Usher syndromes (USH) are a group of genotypically distinct diseases that share several phenotypic characteristics and are recognized as the most frequent cause of hereditary deafness-blindness in humans. These syndromes are characterized by autosomal recessive inheritance and by dual sensory impairments of the inner ear

and the retina (Usher 1914). Variations in severity of hearing loss and vestibular response among affected families distinguish between three different Usher phenotypes, known as Usher type 1 (USH1), Usher type 2 (USH2), and Usher type 3 (USH3) (Moller et al. 1989; Smith et al. 1992b; Sankila et al. 1994). On the basis of phenotypic differences, genetic heterogeneity was hypothesized (Davenport and Omenn 1977) and has been verified recently by linkage studies. USH1 shows linkage to at least five different loci: chromosome 14q (USH1A; Kaplan et al. 1992), chromosome 11q (USH1B; Kimberling et al. 1992), chromosome 11p (USH1C; Smith et al. 1992a), chromosome 10q (USH1D; Wayne et al. 1996), and chromosome 21q (USH1E; Chaib et al. 1997). USH1B is the most common subtype, accounting for ~70% of all type 1 cases. The myosin VIIA gene was determined to be responsible for USH1B, on the basis of the detection of nonsense and missense mutations and two small deletions in the head region of the gene in three affected families (Weil et al. 1995). Similarly, mutations in the murine myosin VIIA gene were shown to be responsible for the shaker-1 phenotype (Gibson et al. 1995). Immunolocalization showed that the myosin VIIA protein is expressed within tissues involved in the Usher syndrome pathology: inner and outer hair cells of the organ of Corti of the guinea pig and retinal pigment epithelium (RPE) in adult rat (Hasson et al. 1995). A species-specific cell pattern of gene expression was suggested as a possible cause for the discrepancy between the human USH1B and the mouse shaker-1 phenotypes, which both manifest themselves by cochlear and vestibular dysfunction and differ between each other in retinal dystrophy, which is absent in the mouse shaker-1 phenotype (El-Amraoui et al. 1996). Screening of the first 14 exons of the human myosin VIIA yielded 14 mutations, of which 12 were novel, in 21 different families (Weston et al. 1996; Liu et al. 1997). Another screening, covering 48 of the gene's 49 exons, detected eight mutations, of which four were novel, in six different families (Levy et al. 1997). This study reports the results of USH1B haplotypes and mutation characterization in all 49 exons of the myosin VIIA gene, among 15 USH1B cases from eight different ethnic origins (see the appendix).

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## Subjects and Methods

### Subjects

Altogether, 54 affected individuals representing 28 different USH1 families were referred to us through the Center for Deaf-Blind persons in Tel-Aviv, as part of a larger study on the genetics of Usher syndrome in Israel. Patients were considered as Usher type 1 on the basis of the presence of congenital severe hearing loss, vestibular areflexia, and retinal degeneration. Clinical confirmation of this diagnosis was available for only 11 families. The origins of the 28 families are Algerian (two), Bulgarian (one), Hungarian (three), Iranian (two), Moroccan (seven), Romanian (two), Russian (two), Samaritan (one), Tunisian (two), Yemenite (one), Hungarian-Bulgarian (one), Polish-German (two), Polish-Latvian (one), and Romanian-Russian (one).

### Haplotype Analysis

Haplotypes were derived by use of the following polymorphic markers: D11S787, D11S527, OMP, D11S1789, D11S906, and D11S4186 (listed according to their physical order, from the centromeric to the telomeric end), spanning the USH1B locus. Genomic DNA extracted from blood of affected individuals and their family members was used as template. PCR amplification of these markers was performed by use of pairs of specific primers as described by Sambrook et al. (1989). Alleles of each marker were numbered according to their relative mobility on a denaturing formamide/4% acrylamide gel. Allele sizes were determined by comparing their mobility through Long Ranger™ gel (FMC Bio-products) with the mobility of a known DNA fragment.

### Mutation Detection

**SSCP screening.**—Genomic DNA extracted from blood of affected individuals and their family members was screened for mutations within the myosin-VIIA gene, by the use of SSCP analysis (Sheffield et al. 1993) of individually amplified exons. The screen included 49 exons covering the entire open reading frame of the gene and 5' and 3' UTRs of the cDNA. All PCR products were amplified from genomic DNA in the presence of radioactive end-labeled primers, under different PCR conditions as calibrated for each exon. These products were electrophoresed through MDE™ (AT Biochem) gels containing 10% glycerol, at 800 V for 16–20 h. Nucleotide sequences of primers used for the amplification of the myosin VIIA exons were as described by Weston et al. (1996), for exons 1–14, and as described by Levy et al. (1997), for exons 15–49 (which, in Levy et al.'s study, are referred to as exons 14–48, respectively).

**DNA sequencing.**—In order to define mutations/sequence changes, DNA fragments that exhibited mobility shifts in SSCP analysis, as well as normal fragments, were reamplified, gel purified by use of the Qiagen gel

extraction kit, and used as templates in a direct cycle-sequencing reaction by FMC's SequiTherm EXEL™ DNA sequencing kit. Sequencing products were subjected to comparative electrophoresis through FMC's Long Ranger™ gel. Comparative electrophoresis in which normal and shifted sequencing products are electrophoresed side by side (normal ddGTP product next to shifted ddGTP product, normal ddATP product next to shifted ddATP product, etc.) enables a fast and easy detection of homozygous and heterozygous sequence changes.

**ASO analysis.**—When a change was found in a DNA sample from a patient, its occurrence was determined among >100 unrelated individuals by use of the allele-specific oligonucleotide (ASO) hybridization technique as described by Whitney et al. (1993).

**Southern blot analysis.**—Genomic DNAs of affected individuals in whom no mutation was detected by SSCP analysis were digested separately by several restriction enzymes, electrophoresed through 0.8% agarose gel, and transferred to Qiagen nylon membrane. Filters were hybridized overnight at 65°C with four P<sup>32</sup>-labeled probes comprising four different myosin VIIA exons, were washed at 60°C, and were autoradiographed at –80°C by use of x-ray films.

## Results

### Haplotype and Linkage Analyses

Haplotypes comprising six markers listed above were characterized in all affected and normal chromosomes studied in this work. In the Samaritan kindred, linkage analysis yielded a high maximum LOD score of 11.61 (Bonne-Tamir et al. 1994), and, in five other families, linkage analysis yielded maximum LOD scores of 1.06–2.36. In other families, linkage results were limited because of the small number of members available for typing. USH1B haplotypes presented in table 1 include haplotypes of chromosomes in which myosin VIIA mutations were detected, except for haplotype 13, which was carried by two affected brothers who are compound heterozygotes for a paternal chromosome represented by haplotype 12. Haplotype 13 represents two chromosomes, one recombinant and one original USH1B maternal chromosome. Markers and intragenic polymorphism similarities indicate that the recombination event in one of these two haplotypes had occurred in the chromosomal interval between the myosin VIIA gene and the OMP locus. Haplotypes 1–11 and 16 were found in a homozygous state because of the consanguineous nature of their pedigrees. Haplotype 4 was found in three unrelated Jewish families, all of whose parents came from the same town in Morocco. This haplotype and its variants, represented by haplotypes 1–5, seem to be the most common (50%) among northern African USH1B chromosomes tested in this work.

**Table 1****Haplotypes of USH1B Chromosomes**

HAPLOTYPE <sup>a</sup>	ALLELE OF MARKER <sup>b</sup>							ORIGIN(S)
	D11S787	D11S527	OMP	(Myosin VIIA) <sup>c</sup>	D11S1789	D11S906	D11S4186	
1	8	2	3		4	2	2	Algeria
2	8	2	3		4	2	1	Algeria, Morocco
3	8	6	3		4	2	2	Algeria
4 <sup>d</sup>	8	6	3		4	2	1	Morocco
5	7	7	4		4	2	1	Morocco
6	9	5	4		3	2	6	Morocco
7	7	3	4		3	2	6	Morocco
8	5	9	3		3	2	6	Morocco
9	6	8	1		5	2	6	Tunis
10 <sup>e</sup>	8	1	1		5	2	10	Tunis
11	4	5	3		8	2	10	Iran
12	6	3	3		8	2	10	Bulgaria
13	6/5	3/7	3/4		5	2	10	Hungary
14	7	0	1		3	2	6	Poland
15	7	0	1		3	2	8	Latvia
16	5	7	1		5	4	5	Samaria

<sup>a</sup> Extra spaces between rows have been used to group haplotypes in which the same mutation was detected.

<sup>b</sup> Sizes of specific alleles listed in this table are as follows: D11S787 (size range of known alleles is 168–184 bp) 4 = 178 bp; 5 = 176 bp; 6 = 174 bp; 7 = 172 bp; 8 = 170 bp; and 9 = 168 bp; D11S527 (range 144–162 bp) 0 = 162 bp; 1 = 160 bp; 2 = 158 bp; 3 = 156 bp; 5 = 152 bp; 6 = 150 bp; and 7 = 148 bp; OMP (range 256–262 bp) 1 = 262 bp; 3 = 258 bp; and 4 = 256 bp; D11S1789 (range 238–258 bp) 3 = 254 bp; 4 = 252 bp; 5 = 250 bp; and 8 = 244 bp; D11S906 (range 287–295 bp) 2 = 293 bp and 4 = 289 bp; and D11S4186 (range 165–175 bp) 1 = 174 bp; 2 = 173 bp; 5 = 170 bp; 6 = 169 bp; 8 = 167 bp; and 10 = 165 bp.

<sup>c</sup> According to a Yac contig constructed in our laboratory (Seroussi et al. 1994), the position of the myosin VIIA gene was established as being between markers OMP and D11S1789.

<sup>d</sup> Haplotype 4 was found in three unrelated Jewish families, all parents of which came from the same town in Morocco.

<sup>e</sup> Haplotype 10 is from a non-Jewish family that resides in Tunisia.

**Mutation Screening**

Mutation screening was performed on members of all 28 USH1 families studied in this work. Eight novel mutations were identified in a total of 42 affected individuals from 15 families (see appendix), verifying their classification as USH1B. Table 2 summarizes the mutations observed in this study. Codon numbering starts with the first in-frame methionine of the human myosin VIIA (Weil et al. 1996).

**A. Missense mutations.**—Altogether, three missense mutations were identified among carriers and affected in nine unrelated families: Gly→Arg in exon 7, Ala→Asp in exon 11, and Ala→Thr in exon 21. These missense mutations were found in the homozygous state as follows: 1 patient of Moroccan origin was homozygous for the Gly→Arg substitution in exon 7, 2 affected brothers of Iranian origin were homozygous for the Ala→Asp mutation in exon 11, and 13 patients from six different families of Moroccan and Algerian origins were homozygous for the Ala→Thr mutation in exon 21. These

mutations were found in the heterozygous state as follows: two affected brothers of Bulgarian-Hungarian origin were heterozygous for the Ala→Asp mutation in exon 11, and another patient of Moroccan origin, a cousin of the homozygote for the missense mutation in exon 7, was a compound heterozygote for the Gly→Arg and Ala→Thr substitutions in exons 7 and 21, respectively. The Gly→Arg mutation in exon 7 and the Ala→Thr mutation in exon 21 were not observed among >200 control chromosomes tested by ASO analysis for the presence of these mutations. The Ala→Asp mutation in exon 11 was observed only in 1 of >200 control chromosomes tested by ASO analysis.

**B. Nonsense mutation.**—One mutation that leads to a premature stop codon was found in a homozygous state in 16 affected members of one large Samaritan kindred described by Bonne-Tamir et al. (1994). This is a C→T transition at codon 1861 in exon 40, causing an Arg→stop codon (CGA→TGA), which would result in a truncated protein that lacks >25% of tail domain.

**Table 2****Summary of Myosin VIIA Mutations Detected in USH1B Patients**

Mutation	Exon(s)	Nucleotide Change	Genotype(s)	Haplotype(s)	Origin(s)
Missense:					
Gly214Arg	7	GGA→AGA	homozygote, Compound heterozygote	8	Morocco
Ala397Asp	11	GCC→GAC	homozygote, Compound heterozygote	11, 12	Bulgaria, Iran
Ala826Thr	21	GCC→ACC	homozygote, Compound heterozygote	1, 2, 3, 4, 5	Algeria, Morocco
Nonsense:					
Arg1861stop	40	CGA→TGA	homozygote	16	Samaria
Deletion:					
2065delC	45	delCCC	homozygote	6, 7	Morocco
2119–2215del2kb <sup>a</sup>	47–49	>2-kb deletion	homozygote	9	Tunis
Splicing:					
IVS5+1g→a <sup>b</sup>	5	AGgtg→AGatg	homozygote	10	Tunis
IVS18+1g→a <sup>b</sup>	18	AGgtg→AGatg	homozygote	14, 15	Poland, Latvia

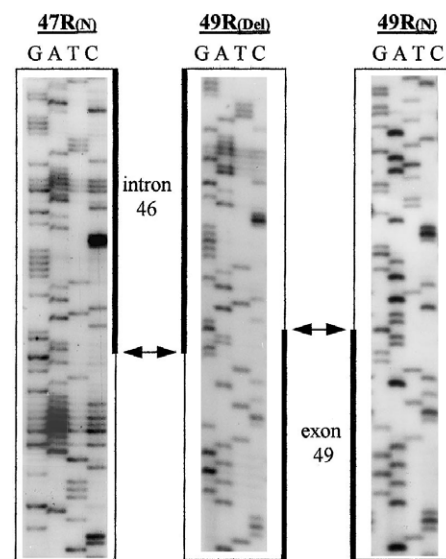
<sup>a</sup> del2kb = large genomic deletion including intronic sequences.

<sup>b</sup> IVS = intronic splicing mutation.

*C. Deletions.*—Two different deletions were identified among members of three unrelated families. One deletion, which is a single cytosin deletion at codon 2065 in exon 45, was found in a homozygous state in three patients from two families of Moroccan origin. The second was a large genomic deletion of >2 kb, including all the translated part of exons 47–49 and the introns between them. This deletion was found in a homozygous state in two affected sisters of a Jewish Tunisian family and in a heterozygous state in their parents and children. A 1.4-kb deleted fragment was PCR amplified from both affected sisters, by use of the forward primer of exon 46 and a reverse primer derived from the internal sequence of exon 49. This deletion fragment was reverse sequenced and compared with the reverse sequence of two fragments amplified from normal controls: one fragment including exon 49 and another fragment including exons 46–47. As shown in figure 1, this comparison enabled the determination of the deletion borders on the genomic sequence. The deletion starts ~20 nucleotides upstream from the beginning of exon 47 and ends at nucleotide 7079, which is 249 nucleotides downstream from the beginning of exon 49, including its entire coding region. Sequencing results revealed two different small repeats (TGGGCC and TGATG) in the normal genomic sequence near both edges of the deletion: three nucleotides downstream from the edge in the end of intron 46 and three nucleotides upstream from the edge in exon 49. Only one copy of these repeats is present in the deleted fragment.

*D. Splice-site mutations.*—Two splice mutations were detected among members of two unrelated families. The first is a G→A transition at position +1 (the first intronic nucleotide in the splice-donor site of exon 5). This mutation was observed in a homozygous state in two siblings of one family of non-Jewish Tunisian

origin. The second splice mutation is also a G→A transition, at position +1 in the splice-donor region of exon 18. This mutation was observed in a homozygous state in one patient of a nonconsanguineous family in which one parent originated from Poland and the other originated from Latvia. These mutations were not observed among >200 control chromosomes tested by ASO analysis.



**Figure 1** PCR cycle-sequencing results for three different DNA fragments: exon 47 PCR product amplified from genomic DNA of a normal individual (47R<sub>(N)</sub>), deleted exon 49–exon 46 PCR product amplified from genomic DNA of an affected individual from the Jewish Tunisian family (49R<sub>(Del)</sub>), and exon 49 PCR product amplified from genomic DNA of a normal individual (49R<sub>(N)</sub>). These DNA fragments were sequenced by use of the reverse primers of exons 47 and 49. Arrows indicate the end of homology, which continues along the thicker lines between the 2-kb deleted fragment and the normal sequence.

In 13 of the 28 USH1 families included in this study no myosin VIIA mutations were detected. Several Southern blot analyses were performed by use of various restriction enzymes, in order to detect the presence of hemizygous large deletions that are undetectable by SSCP screening. At present, no such deletions have been identified.

**E. Polymorphisms.**—Several base substitutions (exonic and intronic), one small intronic deletion, and one small intronic insertion were detected and defined by SSCP and sequencing analyses, in both affected and control samples (table 3). Some shifts observed by SSCP were not sequenced to determine the nature of their change; therefore, there are more polymorphisms than those listed in table 3. Polymorphisms detected in several different chromosomes are referred to as “common” in the origin category of table 3. Two amino acid changes, I205V and I1954L in exons 7 and 43, respectively, are rare and segregate with USH1B chromosomes. However, both changes are conservative; in the case of I205V, the change increases homology to the conserved heptapeptide of human and pig myosin VI and to the myosin II consensus (Weil et al. 1995), and the I1954L change is found in *cis* with the R1861X nonsense mutation. Two intronic substitutions, IVS12+8g→a and IVS16+14gg→aa (in introns 12 and 16, respectively), are also rare and segregate with the disease chromosomes of USH1 families in which no myosin VIIA mutations were detected. However, these changes do not oc-

cur in consensus splice sites; they are not analogous to any splice mutations known in the literature, and cDNA analysis was not carried out. Therefore, we considered these two intronic changes as polymorphisms. The common deletion of 22 nucleotides (ggaggcggggacaccagg-gcct) observed in the intronic region following exon 27 might be associated with the case of alternative splicing products that share the first 27 exons of the large transcript reported by Chen et al. (1996) and Kelley et al. (1996).

## Discussion

In this study, we have characterized USH1B haplotypes and mutations among a relatively small number of families, most (13 of 15) of whom are Jewish. In diseases such as USH1B, in which the responsible gene has already been identified, haplotype analysis provides an additional means for the characterization of disease chromosomes and mutations. An example of this is the case of haplotype 1, in which the SSCP results were not clear enough to indicate the presence of mutation in any of the 49 screened exons; however, the close homology with haplotype 2 (in two other families, one of which was of the same ethnic origin) led us to sequence and detect the same mutation in exon 21. Most haplotypes constructed are population specific. Common mutations and partial similarities between haplotypes were observed in individuals from the same or geographically

**Table 3**  
Summary of Myosin VIIA Polymorphisms

Polymorphism	Exon-Specific PCR Product	Nucleotide Change	Origin(s) <sup>a</sup>	Frequency <sup>b</sup>
Ile156Ile	5	ATC→ATT	Morocco, Samaria	2/55
Ile205Val	7	ATC→GTC	Russia	1/55
IVS12+8g→a <sup>c</sup>	12 <sup>d</sup>	CAGgt accgc(g→a)tg	Romania	1/55
IVS16+14gg→aa <sup>c</sup>	16	ATGgt...cct(gg→aa)ggt	Hungary	1/55
IVS27+10del22nucleotides <sup>c</sup>	27	CCGgt...cg(Δgg...ct)ga	Common	20/55
Ser1358Ser	31	TCC→TCT	Bulgaria, Iran	2/55
IVS32+34g/t <sup>c</sup>	32	AAGgt...gat(g/t)tg	Common	21/55
Ser1585Ser	35 <sup>e</sup>	AGT→AGC	Common	15/55
IV36+21c→t <sup>a</sup>	36	GTGgt...gtgg(c→t)aga	Romania	1/55
Lys1905Lys	41 <sup>e</sup>	AAG→AAA	Common	20/55
Ile1954Leu	43	ATC→CTC	Samaria	1/55
IVS42-7a/t <sup>c</sup>	43	tg(a/t)ccccagGTC	Common	23/55
IVS42-26ins ttgag <sup>c</sup>	43	ag(→ttgag)gt...cagGTC	Russia	2/55
Ser2080Ser	46 <sup>e</sup>	TCC→TCT	Common	3/55
Lys2106Lys	46 <sup>e</sup>	AAA→AAG	Common	40/55
Asn2173Asn	48	AAC→AAT	Common	3/55
IVS48+25a/g <sup>c</sup>	48	CTGgt...gcct(a/g)gtg	Common	3/55

<sup>a</sup> Common polymorphisms found in chromosomes of three or more different origins.

<sup>b</sup> Estimated from 55 unrelated chromosomes included in SSCP screening.

<sup>c</sup> IVS = intronic splicing mutation.

<sup>d</sup> Reported by Weston et al. (1996).

<sup>e</sup> Reported by Levy et al. (1997).

close origins. One partial similarity was also observed between two haplotypes of diverse origins, one from Bulgaria and the other from Iran. All these similarities can be explained by ancestral recombination events and, possibly, by migration patterns of Jewish communities. Haplotypes that shared the same mutation—haplotypes 1–5, haplotypes 6 and 7, haplotypes 11 and 12, and haplotypes 14 and 15—also shared most intragenic polymorphisms and some other allelic similarities. This observation supports the assumption that each of these mutations arose as a single and ancient mutational event, and it suggests that common USH1B alleles can be defined within populations. Such relative homogeneity of USH1B, which was not reflected in findings reported by Weston et al. (1996), is most likely related to the relative isolation of and marriage patterns among Jewish communities.

Mutation analysis detected eight different novel mutations. None of the already known 20 USH1B mutations previously reported were identified among the families included in this work.

These results demonstrate the diversity of mutations responsible for USH1B and can be attributed to differences in mutation spectrum among populations. Patients screened in other studies were of northern and western Europe and American origins, whereas affected individuals studied by us are from eastern Europe, northern Africa, and the Middle East.

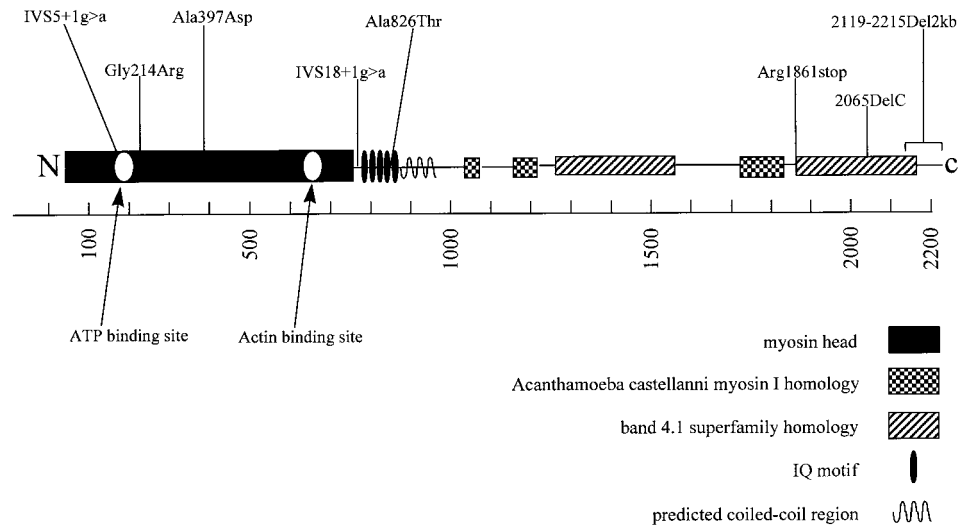
Exploring the location and nature of myosin VIIA mutations might shed more light on both the molecular basis of the USH1B disorder and the role of myosin VIIA in cellular function. Human myosin VIIA is typical of the unconventional-myosins group (Weil et al. 1996). All myosins are actin-based molecular motors that interact with actin filaments, converting energy from ATP hydrolysis into mechanical force used to perform diverse cellular functions. Three different functional domains are defined within the myosin heavy chains: an N-terminal head domain (motor domain) harboring a typical ATP binding site and an actin-binding site, a neck region (regulatory domain) containing at least one IQ motif, and a tail domain that varies dramatically, in both length and sequence, from one unconventional myosin to another and that is thought to be a specific membrane binder (Cheney et al. 1993; Titus 1997). A schematic distribution of mutation (detected in this study) along the human myosin VIIA gene is shown in figure 2.

Three of the eight novel mutations are located in the motor (head) domain. The first is the IVS5+1g→a transition, which lies in the splice-donor site of exon 5. The great majority of point mutations identified within donor splice-sites (ss) occur in the +1G residue, and in 64% of these cases the substituting base is an adenosine (Krawczak et al. 1992). This IVS5+1g→a transition is expected to result either in exon skipping or in frameshift due to cryptic splice-site usage. Both possibili-

ties would seriously impair the proper construction of an affective myosin VIIA mRNA. The second head mutation is the Gly214Arg substitution in exon 7, which occurs within a highly conserved heptapeptide sequence that is invariant in all myosins of all classes (Weil et al. 1995). This mutation resides C-terminal to the ATP binding loop, at the base of the ATP binding pocket. In light of the absolute evolutionary conservation of residues within this region, it is expected that the Gly214Arg mutation would severely impair the ATP-binding function of the protein. The third head mutation is the Ala397Asp substitution in exon 11, which lies between the ATP- and the actin-binding sites within a relatively nonconserved region of the protein. However, this substitution was found in a homozygous state in USH1B-affected individuals, and, in addition, it was shown to be very rare, occurring in only one control chromosome (of Iranian origin) of >200 tested by ASO analysis. In this substitution, an alanine, which is a small and simple amino acid with an aliphatic residue, is replaced by aspartate, which is a larger amino acid with a negatively charged residue. Such a change might alter the three-dimensional structure of the N-terminal domain and hence might have a crucial effect on the function of the protein motor domain.

The head domain of human myosin VIIA is followed by a neck (regulatory) region containing five consecutive repeats of the IQ motif (Weil et al. 1996). Two of the eight mutations reported in this work are located in the neck domain. The first is IVS18+1g→a, which lies in the splice-donor region of exon 18. This transition, as has been described for the one discussed above, is expected to cause a splicing defect, which would seriously impair the proper construction of an affective myosin VIIA mRNA. The second neck mutation is the Ala826Thr substitution in exon 21, which lies within the C-terminal part of the myosin VIIA fourth IQ motif (in a series of five). This motif is one of the three motifs that match the consensus sequence of calmoduline-binding IQ motifs (Wolenski 1995). Thus, the Ala→Thr change in codon 826 is expected to impair the calmoduline chain-binding function of the protein.

The human myosin VIIA possesses the longest tail (1,360 amino acids) of all unconventional myosins identified so far (Weil et al. 1996). Three mutations, all of which are predicted to result in a truncated protein, were located in the tail domain. The first is the Arg1861stop mutation in exon 40. This nonsense mutation would result in a truncated protein that lacks >25% of the normal tail domain, including its C-terminal part. The C-terminal part of myosin-VIIA is hydrophilic, more basic than the rest of the tail, and shares homology with the membrane-binding domain of various members of the band-4.1 protein superfamily (Rees et al. 1990; Takeuchi et al. 1994). Therefore, the Arg1861stop mutation is predicted to impair the membrane-binding func-



**Figure 2** Schematic representation of the relative linear location of each mutation along the myosin VIIA gene. The gene structure is according to Chen et al. (1996) and Weil et al. (1996). “2119–2215Del2kb” denotes a large (2-kb) genomic deletion.

tion of the protein. The second tail mutation is a single cytosine deletion at codon 2065 in a CCCC string in exon 45, which also encodes a part of the second region homologous to the band-4.1 protein superfamily in the myosin VIIa tail. This deletion is expected to cause a frameshift that will lead to the formation of a UAA stop codon 64 amino acids downstream from the deletion and would therefore result in a truncated protein that lacks the last 214 amino acids at the C-terminal end. Such a defect is expected to impair the membrane-binding function of the protein, as described above. The third tail mutation is a large genomic deletion that includes exons 47, 48, all the translated part of exon 49, and the introns between them. This deletion spans the last third (96 amino acids) of the second region homologous to the band-4.1 protein superfamily and thus is also expected to have an impairing effect on the membrane-binding function of the protein. The two small repeats (TGGGCC and TGATG) that were found near the edges of this deletion in the normal genomic sequence could have been involved in recombination and loop-fragment excision, leading to the formation of this deletion.

No significant difference was found in mutation distribution among the three defined domains of the human myosin VIIA gene. This result does not agree with the Weston et al.’s (1996) expectation that the majority of mutations would be found in the tail domain. Furthermore, when also considering the mutations described by Levy et al. (1997), we get the following distribution: 8:2:6, for head, neck, and tail domains, respectively. In view of the fact that the tail segment of the human myosin VIIA consists of more than half the gene (Weil et al. 1996), such a distribution actually shows that the majority of mutations are located within the head do-

main. These results are consistent with the mutation distribution described for the b-cardiac myosin II (Rayment et al. 1995).

The mutations described were observed in a total of 42 affected individuals from 15 USH1B families, including 1 singleton case. In 14 of these families, mutations have been found in both alleles. Most affected individuals were homozygous for the same mutation, except for one patient who was a compound heterozygote for two different mutations. In three additional USH1 families and in 10 USH1-singleton cases, no myosin VIIA mutations were detected. None of these cases are consanguineous, and, in four of them, only a partial haplotype homozygosity has been found in the myosin VIIA locus. In these three families (each of which includes one affected child and two or three healthy siblings), haplotype analysis suggested linkage to the USH1B locus, with a relatively low LOD score due to the small number of members in each family. In all the singleton cases, as well as in the three small families, the possible involvement of another USH1 locus cannot be ruled out. However, even if these cases are actually linked to the USH1B locus, undetected myosin VIIA mutations might be located within uncharacterized regions of the gene, including the promoter region, unexplored intron sequences, and unexplored 3’ and 5’ UTRs. Another possible explanation for the lack of detection of mutations might be the limitations of the SSCP technique. One of these limitations is that SSCP cannot detect the presence of relatively large hemizygous deletions, as were observed in a homozygous state in the Jewish Tunisian family presented in this work. Southern blot analysis performed in an attempt to overcome this limitation has not yet revealed any such deletions.

## Appendix

**Table A1**

Summary of Data and Results for All USH1B Families

Family	Ethnic Origin	No. of Affected Individuals	Consanguinity <sup>a</sup>	Haplotype(s)	Genotype(s)	Mutation
1	Algeria	1	+	1	Homozygote	Ala826Thr
2	Algeria, Morocco	1	–	1, 2	Heterozygote	Ala826Thr
3	Bulgaria, Hungary	2	–	12 13	Heterozygote	Ala397Asp Unknown
4	Iran	2	++	11	Homozygote	Ala397Asp
5	Latvia, Poland	4	–	14, 15	Heterozygote	IVS18+1g→a
6	Morocco	4	++	2	Homozygote	Ala826Thr
7	Morocco	2	+	3	Homozygote	Ala826Thr
8	Morocco	2	+	4	Homozygote	Ala826Thr
9	Morocco	2	++/–	4	Homozygote	Ala826Thr
10	Morocco	2	+++/–	8	Homozygote, Heterozygote	Gly214Arg
11	Morocco	2	+++	4 6	Heterozygote Homozygote	Ala826Thr 2065delC
12	Morocco	1	++	7	Homozygote	2065delC
13	Samaria	16	++	16	Homozygote	Arg1861stop
14	Tunis	2	–	9	Homozygote	2119–2215del2kb <sup>b</sup>
15	Tunis	2	++	10	Homozygote	IVS5+1g→a

<sup>a</sup> + = second-cousin marriage; ++ = first-cousin marriage; +++ = uncle-niece marriage; and – = no consanguinity.

<sup>b</sup> IVS = intronic splicing mutation.

<sup>c</sup> del2kb = large genomic deletion including intronic sequences.

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