The Usher Syndromes

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Mutations in the gene (MYO7A) encoding myosin-VIIa, a member of the large superfamily of myosin motor proteins that move on cytoplasmic actin filaments, and in the USH2A gene, which encodes a novel protein resembling an extracellular matrix protein or a cell adhesion molecule, both cause Usher syndrome (USH), a clinically heterogeneous autosomal recessive disorder comprising hearing and visual impairment. Patients with USH1 have severe to profound congenital hearing impairment, vestibular dysfunction, and retinal degeneration beginning in childhood, while those with USH2 have moderate to severe hearing impairment, normal vestibular function, and later onset of retinal degeneration. USH3 is characterized by progressive hearing loss and variable age of onset of retinal degeneration. The phenotype resulting from MYO7A and USH2A mutations is variable. While most MYO7A mutations cause USH1, some cause nonsyndromic hearing impairment, and one USH3 phenotype has been described. USH2A mutations cause atypical USH as well as USH2. MYO7A is on chromosome region 11q13 and USH2A is on 1q41. Seven other USH genes have been mapped but have not yet been identified. USH1A, USH1C, USH1D, USH1E, and USH1F have been assigned to chromosome bands 14q32, 11p15.1, 10q, 21q21, and 10, respectively, while USH2B is on 5q, and USH3 is at 3q21-q25. Myosin Vlla mutations also result in the shaker-1 (sh1) mouse, providing a model for functional studies. One possibility is that myosin-VIIa is required for linking stereocilia in the sensory hair bundle; another is that it may be needed for membrane trafficking. The ongoing studies of myosin-Vlla, the USH2A protein, and the yet to be identified proteins encoded by the other USH genes will advance understanding of the Usher syndromes and contribute to the development of effective therapies.


KEY WORDS: hearing impairment; pigmentary retinopathy; myosin Vlla; genetic heterogeneity; clinical heterogeneity

INTRODUCTION

The Usher syndromes (USH) are a group of clinically variable and genetically heterogeneous autosomal recessive disorders. They are characterized by congenital sensorineural hearing loss and pigmentary retinopathy, which usually manifests in late childhood or adolescence and may lead to total blindness. Unless there is an affected relative, a child may be diagnosed as having a nonsyndromic congenital hearing impairment, with USH not being considered until the onset of vision loss in the second decade. The prevalence of USH is estimated to be between 1/16,000 and 1/50,000 based on studies in Scandinavia [Hallgren, 1959; Nuutila, 1970; Grondahl, 1987; Rosenberg et al., 1997], Colombia [Tamayo et al., 1991], the United Kingdom [Hope et al., 1997], and the United States [Boughman et al., 1983]. USH is reported to account for between 3% and 6% of the congenitally deaf population [Vernon, 1969], about 18% of those with retinitis pigmentosa, and more than 50% of the deaf-blind population [Boughman et al., 1983]. The significance of advancing understanding of the genetic defects responsible for the single, major cause of deaf-blindness and relieving the tremendous personal burden imposed by loss of both vision and hearing cannot be underestimated.

The German ophthalmologist Albrecht von Graefe described the association of congenital hearing impairment and progressive pigmentary dystrophy of the retina in 1858, and Charles Usher, a British ophthalmologist, provided extensive clinical documentation of affected individuals [Gorlin et al., 1995]. In particular, Usher [1914] emphasized the autosomal recessive pattern of inheritance and suggested the existence of at least two clinical types of USH based on the degree of hearing impairment and the age of onset and progression of the visual loss.

PHENOTYPE

The two types of USH described by Usher [1914] are the forms that are most widely recognized clinically. Patients with Usher syndrome type I (USH1) have severe to profound congenital hearing impairment, vestibular dysfunction, and retinal degeneration beginning in childhood, while those with type II (USH2) have moderate to severe hearing impairment, normal vestibular function, and later onset of retinal degeneration. Detailed diagnostic criteria were defined by the Usher Syndrome Consortium [Smith et al., 1994].
The relative percentages of USH1 and USH2 vary among studies but recent reports suggest that type II is more common than type I [Fishman et al., 1983; Kimberling et al., 1991; Hope et al., 1997; Rosenberg et al., 1997]. A third type of Usher syndrome (USH3) is characterized by progressive hearing loss and variable age of onset of retinal degeneration [Davenport and Omenn, 1983; Kimberling et al., 1991; Hope et al., 1997; Rosenberg et al., 1997]. A comprehensive ophthalmological examination may provide a definitive diagnosis of USH and distinguishes it from nonsyndromic sensorineural hearing impairment. Night blindness is often the first symptom of the retinal degeneration. A comprehensive ophthalmological examination may provide valuable prognostic information [Fishman et al., 1995; Edwards et al., 1996, 1998], although van Aarem et al. [1995c] reported considerable variability in visual acuity among Dutch USH2 patients. Visual loss gradually increases due to progressive degeneration of rod photoreceptor cells, but it may not become a serious problem until the third or fourth decade. However, abnormalities in an electroretinogram (ERG) may be detected in early childhood [Young et al., 1996]. Thus, an ERG should be done at an early age and repeated at regular intervals for any hearing impaired child who may be at risk for USH. Early diagnosis of USH would enable an extended time to prepare for both the physical and emotional impact of the gradual visual loss [Miner, 1995; Tamayo et al., 1997].

Vestibular dysfunction, in addition to degree of hearing impairment, distinguishes USH1 from USH2. Children with USH1 show delayed motor development because they cannot sense gravity [Müller et al., 1989; Smith et al., 1994], and show no nystagmus in response to ice-water caloric stimulation. On the other hand, USH2 patients show normal motor development and brisk nystagmus.

Other clinical findings that have been reported in USH patients include olfactory loss [Zrada et al., 1996], structural abnormalities of nasal cilia [Arden and Fox, 1979; Marietta et al., 1997], and decreased sperm motility [Hunter et al., 1986]. Hunter et al. [1986] also found a high proportion of abnormal axonemes in retinal photoreceptor cells and Berson and Adamian [1992] observed that most of the connecting cilia of photoreceptors in the macula were abnormal. Biochemical studies suggest that USH1 patients have decreased levels of phospholipids in red blood cells and plasma, but USH2 patients have normal levels [Bazan et al., 1986; Maude et al., 1998]. In general, temporal bone histopathological studies of all USH types have shown extensive degeneration of the hair cells of the organ of Corti and spiral ganglion cells with atrophy of the stria vascularis [Belal, 1975; Cremers and Delleman, 1988; Nadol, 1988a,b; Shinkawa and Nadol, 1986; van Aarem et al., 1995a]. Neuroimaging studies showed a significant decrease in intracranial volume and in size of the brain and cerebellum suggesting that the disease process in USH involves the entire brain and is not limited to the posterior fossa or auditory and visual systems [Schaefer et al., 1998].

**Gene Mapping and Identification**

The Hereditary Hearing Loss Home Page [URL: http://dnalab-www.uia.ac.be/dnalab/hhh] provides a compilation of all mapped loci and identified genes for phenotypes involving hearing loss [Van Camp and Smith, 1999]. Syndromic hearing impairment tends to be less genetically heterogeneous than nonsyndromic. However, the number of genes that have so far been mapped for USH1 is six. These six genes are on chromosome arms 14q [Kaplan et al., 1992], 11q [Kimberling et al., 1992], 11p [Smith et al., 1992a], 10q [Wayne et al., 1997], 1q [Chaibi et al., 1997], and on chromosome 10 [Wayne et al., 1997]. USH2 loci have been assigned to chromosome arms 1q [Kimberling et al., 1990, Lewis et al., 1990] and 5q [Pieke-Dahl et al., 1998], and Sankila et al. [1995] localized a gene for USH3 to 3q. Thus, mutations in nine different genes cause USH, and unlinked families of each type suggest at least three more [Pieke Dahl et al., 1993, 1996, 1998].

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**USH1A**

USH1A was mapped to chromosome band 14q32 by analysis of families from the Poitou-Charentes region of France [Kaplan et al., 1992]. The interval containing the gene was refined by Larget-Piet et al. [1994], who also demonstrated genetic heterogeneity among French USH1 families from different geographic regions. A candidate gene, the human homologue of the gene encoding the echinoderm microtubule-associated protein (EMAP), was isolated from the USH1A region but was ex-
USH1B and Myosin-VIIa

After the initial demonstration of linkage of USH1B to markers at 11q13.5 in families from the United States, Sweden, Ireland, South Africa, and Britain [Kimberling et al., 1992; Smith et al., 1992a], Bonné Tamir et al. [1994] refined the candidate region by analysis of a large Samaritan kindred. The physical mapping of the region and identification of mutations in the myosin–VIIa gene (MYO7A) was presented by Weil et al. [1995] who suggested that USH1B accounts for about 75% of USH1 patients.

The USH1B gene encodes the unconventional myosin–VIIa, a member of the large superfamily of myosin motor proteins that move on cytoplasmic actin filaments [Weil et al., 1995]. This gene was identified through studies of the mouse deafness mutant, shaker-1 (sh1). Sh1 homozygotes exhibit circling and hyperactivity, and progressive loss of hearing and balance in the first few postnatal weeks [Deol, 1956]. Histological examination of the inner ear showed progressive degeneration of the hair-cell epithelia of both auditory and vestibular organs, as well as degeneration of the neurons receiving synaptic input and trophic factors from hair cells [Steel and Bock, 1983]. Because USH1B and sh1 had been mapped to a conserved linkage group at human chromosome band 11q13.5 and mouse chromosome 7, respectively, Sh1 and USH1B were hypothesized to be caused by mutations in orthologous genes. This hypothesis was proven to be correct when Gibson et al. [1995] showed that the sh1 gene encodes myosin–VIIa, and Weil et al. [1995] quickly found mutations in human MYO7A in USH1B patients.

Myosin–VIIa was first identified in a human colon carcinoma cell line [Bement et al., 1994], and was found also in hair cells of the amphibian inner ear [Sole et al., 1994]. Cloning of the full-length human and mouse myosin–VIIa cDNAs demonstrated a large protein of 2215 amino acids (Fig. 1A), which can be expressed in several alternative splice forms [Chen et al., 1996, Weil et al., 1996; Mburu et al., 1997]. It shares with other myosins the conserved head domain, which has the ATP- and actin-binding domains. Following the head are five binding sites for regulatory light chains such as calmodulin (IQ motifs), and a short coiled-coil region suggesting that this myosin normally functions as a dimer. Much of the tail of myosin–VIIa bears two large repeats, each containing a MyTH4 domain and a talin-like domain [Chen et al., 1996]. The MyTH4 domain, of about 100 amino acids, is found in several other members of the myosin superfamily, including myosin–XV, which is also associated with deafness [Probst et al., 1998; Wang et al., 1998]. Certain unusual plant kinases also have a MyTH4 domain, suggesting that this may be a cargo-binding domain that can be connected either to actin-based motors (myosin) or microtubule-based motors (kinesin).

The talin-like domain of 300 amino acids is similar to domains in talin, ezrin, moesin and others that are thought to bind to membrane proteins. Both these domains and the location of myosin–VIIa within cells suggest that this myosin may be associated with membranes or vesicles. Finally, there is an SH3 domain between the two repeats, which could be another site for protein–protein interaction.

A large number of MYO7A mutations that cause Usher syndrome or related hearing disorders have been found. Eight different alleles have been identified in the sh1 mouse, with varying degrees of auditory dysfunction. These mutations occur throughout the myosin–VIIa gene, but the alleles with mutations in important conserved domains have the least amount of myosin–VIIa protein and the most severely compromised hair cells, suggesting that nonfunctional myosin–VIIa is more rapidly degraded in cells [Mburu et al., 1997; Hasson et al., 1997a]. Most of the alleles display some disorganization of the sensory hair bundle, and while stereocilia may still show the staircase arrangement of heights, the bundles seem fragmented into patches with a few stereocilia each [Self et al., 1998]. However, even alleles that show no bundle abnormalities and that have adequate receptor potentials can show abnormal cochlear responses and circling behavior [Self et al., 1998], suggesting that the lethality caused by these mutations is not in the hair bundle. In the human myosin–VIIa gene, almost 90 mutations have been found that cause hearing loss [Weston et al., 1996; Adato et al., 1997; Liu et al., 1997a; see summary in Kimberling et al., 1999]. These occur throughout the gene: 37 of the 48 coding exons bear at least one mutation in different families. Most MYO7A mutations cause a typical USH1 phenotype, but some cause DFNB2, a recessive deafness without retinal degeneration, and DFNA11, a dominantly inherited nonsyndromic hearing impairment [Liu et al., 1997b,c; Weil et al., 1997]. In another family, affected children have the clinical phenotype of USH3, but have mutations in MYO7A [Liu et al., 1997]. Perhaps not surprisingly, some mutations seem to impair myosin–VIIa function more than others, and produce disease of varying extent and severity. It is tempting to try to correlate specific mutations in different domains with specific clinical characteristics, but—with the exception of these three phenotypes that differ from typical USH1—it has not yet been possible.

Because of its role in USH1B, myosin–VIIa has received a great deal of attention in recent years. It is expressed in the cochlea and retina, as expected, but also in testis and (to a lesser extent) lung, kidney, intestine and olfactory epithelium [Hasson et al., 1995; Sahly et al., 1997; Wolfrum et al., 1998]. These other organs seem not to be af-
fected by mutations in MYO7A. In all of these epithelial tissues, myosin-VIIa is associated especially with cilia or microvilli of the apical surfaces [Wolfrum et al., 1998].

Within the inner ear, myosin-VIIa is expressed by the sensory hair cells of both auditory and vestibular sensory epithelia (Fig. 1B), but not appreciably by other cells in the inner ear [Hasson et al., 1995, 1997b; Sahly et al., 1997]. The protein is found throughout the cytoplasm of hair cells and the stereocilia of the sensory hair bundles, and is at highest concentration in two places. One (observed primarily in lower vertebrates) is a band within the “ankle” region of the stereocilia at the upper part of the stereocilia taper. Electron microscopic localization of myosin-VIIa in this region demonstrates label between the actin cores and the membranes of stereocilia. Also localized in this region is a set of extracellular filaments termed “ankle links,” which may serve to hold the stereocilia in register. Perhaps myosin-VIIa is the intracellular attachment to the cytoskeleton for these extracellular links [Hasson, 1997]. This proposed function may account for the disorganized hair cell bundles observed in most sh1 mice.

The other concentration is in the “pericuticular necklace,” a ring near the apical surface of the hair cell that is outside the actin-rich cuticular plate but inside the circumferential actin band of the zonula adherens (Fig. 1C). This zone within the hair cells is distinguished by a large number of vesicles, and is thought to be a region of membrane trafficking [Kachar et al., 1997]. Proteins synthesized in the endoplasmic reticulum that are bound for secretion or for the stereocilia must be transported through this zone, and material endocytosed from the apical surface and bound for degradation must pass the opposite way. The presence of domains in myosin-VIIa similar to those that cause membrane association in other proteins suggests that myosin-VIIa may be bound to these vesicles, and may be part of the transport mechanism [Hasson, 1997]. The sh1 mouse provides further support for membrane trafficking as an important function of myosin-VIIa. Whereas wild-type hair cells in culture normally accumulate aminoglycoside antibiotics from the medium, hair cells from a more severe allele of the sh1 mouse do not [Richardson et al., 1997]. Other markers indicate that the first stages of the endocytic pathway are intact in severe sh1 hair cells, suggesting that myosin-VIIa may function in a later stage of endocytosis, or might transport an aminoglycoside receptor to the membrane. It seems most likely that

Figure 1. Domain structure and location of myosin-VIIa. A: Schematic representation of domains. The motor domain binds actin and hydrolyzes ATP. Five IQ domains are thought to bind regulatory light chains. A short predicted coiled-coil region may mediate dimerization. MyTH4 and talin-like domains take up most of rest of the tail, in two large repeats. B: Myosin-VIIa (shaded) occurs in inner (IHC) and outer (OHC) hair cells of the auditory sensory epithelium. C: Highest concentration of m7a in hair cells is found in the pericuticular necklace (pcn) that rings the cuticular plate (cp), and in a band within stereocilia near the ankle links (al).
a defect in membrane trafficking leads to the death of hair cells that ultimately causes deafness.

In the Acadian population, haplotype data are consistent with a single mutation being responsible for all cases of Acadian Usher syndrome type I.

The retinitis pigmentosa that defines USH in humans results from death of photoreceptor cells—particularly rods—in the peripheral retina. In the retina, myosin-VIla is found in two cell types: in the photoreceptors themselves, and in the retinal pigmented epithelium (RPE) cells, which are involved in resynthesizing the chromophore and in phagocytosing spent photoreceptor discs. In the RPE cells, myosin-VIla is specifically in the microvilli, which face the photoreceptors [Hasson et al., 1995; Liu et al., 1997d]. In photoreceptors, there is a high concentration in the connecting cilium—the narrow stalk connecting inner and outer segments—specifically between the microtubules and the membrane [Liu et al., 1997d].

Sh1 mice do not show retinal degeneration, which is perhaps not surprising since the human retinal pathology takes years to be apparent. Nevertheless, there are subtle defects in sh1 retinas. For instance, RPE cells of sh1 mice fail to transport pigment granules into the microvilli [Liu et al., 1999b]. In sh1 but not wild-type photoreceptor cells, the opsin protein can be observed in the connecting cilium, suggesting a failure of transport to the outer segment [Liu et al., 1999b]. A mild defect in transport of outer segment proteins might cause slow accumulation with eventual toxicity to photoreceptors.

While we still have no definitive function for myosin-VIla, and thus no real understanding of the pathology in USH1B, it seems likely that this protein is involved in transport of vesicles or of membrane-associated proteins at apical surfaces of cells. In the inner ear, and to a lesser extent in the retina, failure of transport leads to the death of the sensory cells.

**USH1C**

Acadian families in which members had Usher syndrome were described by Kloepfer et al. [1966]. The phenotype is usually USH1, but two Acadian families with an USH2 phenotype have been described [Smith et al., 1992b]. The original Acadians were French fishermen who left the northern coastal regions of France (Brittany, Normandy) in the early 1600’s and settled in the Canadian territory known as Acadia (now Nova Scotia and surrounding areas). According to Rushton [1979], their population size grew from a few hundred up to nearly 20,000 by 1755 when the English ordered their expulsion from Acadia, an event known as “Le Grand Dérangement des Acadiens”. The Acadians were dispatched to Maryland, the Carolinas, and Georgia as well as to French ports and the West Indies; over the next 40 years about 4,000 Acadians made their way from these places to Louisiana. At first they settled along the banks of the Mississippi River above New Orleans, but with the Louisiana Purchase in 1803 and statehood in 1812 they were forced west across the Atchafalaya Basin, a 20 mile wide, almost impenetrable swamp. They built their houses on the plains among the bayous of southwestern Louisiana and remained relatively isolated because of linguistic, religious, and cultural cohesiveness, as well as geographic isolation.

All of the Acadian USH1 families show linkage to markers on chromosome region 11p15.1 [Smith et al., 1992a]. After refinement of the interval containing USH1C [Keats et al., 1994; Ayyagari et al., 1995], YAC, BAC, and PAC contigs were built across the critical region of approximately 400 kb [Ayyagari et al., 1996; DeAngelis et al., 1998; Higgins et al., 1998]. In the Acadian population, haplotype data are consistent with a single mutation being responsible for all cases of Acadian Usher syndrome type I [Keats et al., 1994]. Thus, for profoundly hearing impaired infants of Acadian ancestry, analysis of markers linked to USH1C may allow differentiation between nonsyndromic hereditary hearing impairment and USH1.

Recently, a Lebanese family linked to the USH1C region was reported by Saouda et al. [1998]. A nonsyndromic hearing impairment locus, DFNB18, has been mapped to the same region [Jain et al., 1998]. These families may be helpful in the final identification of the USH1C gene. The rd5/rd5 mouse mutant has been proposed as a model for USH1C because the regions of mouse chromosome 7 and human chromosome 11 to which they map may share homology [Heckenlively et al., 1995]. The ERG in these mice has reduced amplitudes that are distinguished by six months and hearing thresholds are above those of controls.

**Eudy et al. [1998] showed that the USH2A gene on 1q encodes a novel 1,551 amino acid protein, which resembles an extracellular matrix protein or a cell adhesion molecule.**

**USH1D, USH1E, USH1F**

The loci USH1D, USH1E, and USH1F, were all localized by homozygosity mapping. Wayne et al. [1996] mapped USH1D to chromosome 10q by analysis of a Pakistani family in which the parents were first cousins. The nonsyndromic hearing impairment locus, DFNB12 [Chaib et al., 1996] has been assigned to the same region. A consanguineous Moroccan family provided the data for assigning USH1E to chromosome 21q21 [Chaib et al., 1997], and analysis of an inbred Hutterite family suggested that USH1F is also on chromosome 10 [Wayne et al., 1997], but distinct from USH1D.
USH2A

USH2A was the first Usher locus mapped [Kimberling et al., 1990; Lewis et al., 1990]. The chromosome 1q41 region containing USH2A was narrowed to 2.1 cM by Kimberling et al. [1995], a YAC contig was constructed [Sunneg et al., 1996], and the region was further refined [Bessant et al., 1998; Saouda et al., 1998]. After construction of a BAC contig, Eudy et al. [1998] identified the USH2A gene by detection of mutations in a patient’s DNA. Families unlinked to USH2A suggested a second locus, USH2B, which was recently mapped to chromosome 5q [Pieke Dahl et al., 1998].

Eudy et al. [1998] showed that the USH2A gene on 1q encodes a novel 1,551 amino acid protein, which resembles an extracellular matrix protein or a cell adhesion molecule. It contains both laminin epidermal growth factor and fibronectin type II motifs. Similar proteins are known to influence neuronal-glial interactions and to be involved in synapse development and stabilization. USH2A expression was detected in human fetal cochlea, eye, brain and kidney, and in adult retina. Three different mutations were found, with one of them (2314delG) appearing to be relatively frequent in affected individuals of northern European ancestry. A follow-up study of USH2 and atypical USH families (USH3-like) from the United Kingdom and China showed that both phenotypes may result from homozygosity for the 2314delG mutation [Liu et al., 1999a].

USH3

The USH3 locus was mapped to the chromosome region 3q21-q25 in Finnish families [Sankila et al., 1995] and refined to a 1 cM interval [Joensuu et al., 1996]. Gasparini et al. [1998] presented an Italian family showing linkage to markers in this interval, suggesting that this genetic form of USH3 is not restricted to the Finnish population.

DISCUSSION

Although clinical variability had been extensively documented, it was not until Kimberling et al. [1990] and Lewis et al. [1990] found linkage to chromosome 1 markers in USH2 families but not in USH1 families that genetic heterogeneity was demonstrated. Rapid progress has been made in advancing our understanding of the genetics of the Usher syndromes over the past 10 years. The initial impetus was the formation of the Usher Syndrome Consortium, which brought together investigators from around the world with an interest in identifying genes for the Usher syndromes. This was possible because of the support of NIDCD and the Foundation Fighting Blindness.

Mapping studies have localized nine USH genes through linkage to genetic markers. As well as delineating the region containing the gene, these closely linked markers provide data for determining if a relative of an affected member is a carrier, and for an endogamous population such as the Acadians the marker information can be used to calculate the probability that any member of the population is a carrier. Some studies suggest that audiometric and electrooculographic analyses may also be helpful for detecting carriers, although results are inconclusive [van Aarem et al., 1995b; Meredith et al., 1992; Wagenaar et al., 1995, 1996]. However, detailed otoacoustic emission testing of Ashkenazi Jewish carriers of the 167delT mutation in the connexin-32 gene (GJB2) suggested differences in emission patterns between those who carry the mutation and those who do not [Morell et al., 1998].

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