CMLS, Cell. Mol. Life Sci. 56 (1999) 258–267
1420-682X/99/040258-10 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Molecular genetics of Usher syndrome

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Received 9 February 1999; received after revision 15 April 1999; accepted 21 April 1999

Abstract. The Usher syndrome, an autosomal recessive deafness and blindness, is genetically and clinically heterogeneous. In the past 4 years, genes mutated in Usher syndrome type Ib and type IIa have been described. The Usher Ib gene encodes the motor protein myosin VIIa and was identified as the human homolog of the mouse *shaker-1* gene. The Usher type IIa gene was identified by positional cloning and encodes a protein with homology to extracellular matrix proteins and cell adhesion molecules. This review summarizes the current knowledge regarding both the genetic and molecular aspects of Usher syndrome in the context of recent scientific advances in the areas of sensorineural deafness and retinitis pigmentosa.

Key words. Usher syndromes; retinitis pigmentosa; deafness; extracellular matrix; myosin.

Introduction

Usher syndrome comprises a group of autosomal recessive disorders characterized by congenital sensorineural hearing loss, vestibular dysfunction and progressive retinitis pigmentosa (RP) [1]. Von Grafe was the first to recognize the simultaneous occurrence of deafness and RP in 1858 [2], and in 1935 Usher described the hereditary nature of the syndrome that was named after him [3]. The syndrome is the foremost cause of combined blindness and deafness in the industrialized world. Its prevalence in developed countries is estimated at approximately 4/100,000; in addition, it has been estimated that up to 8–33% of individuals with RP also have some degree of hearing loss and that many of these individuals may be suffering from Usher syndrome [4]. As the loss of vision and hearing is especially compromising to the individuals affected, their family and society as a whole, insight into the molecular aspects of the disease is crucial to the development of treatment.

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Substantial progress has been made in the last 2 decades with respect to the understanding of Usher syndrome. It has become apparent that the syndrome is both clinically and genetically heterogeneous. Whereas all Usher patients suffer progressive RP, the degree of hearing impairment and the presence or absence of vestibular abnormalities differ between the subtypes [5–11]. For these reasons, Usher syndrome has been divided into three clinical subtypes: Usher syndrome type I (USH1), Usher syndrome type II (USH2) and Usher syndrome type III (USH3). USH1 is characterized by profound congenital deafness, vestibular areflexia and prepubertal onset of progressive RP. USH2 is characterized by moderate to severe congenital deafness, normal vestibular responses and adolescent onset of progressive RP. USH3 is characterized by progressive hearing loss, variable vestibular responses and adolescent onset RP.

The study of Usher syndrome has a major impact, not only on the field of research of blindness and hearing loss but also on the understanding of fundamental principles in the basic molecular biology of vision and hearing. The phenotypes of patients with mutations in

these two genes will be of importance for evaluating the role of motor proteins and cell adhesion molecules in sensorineural processes. Additionally, recent identification of Usher Ib and Usher IIa genes opens up the possibility of developing therapies for this disease [12, 13].

In this review, we summarize the current knowledge in the field of Usher syndrome and discuss it in the context of the recent developments. Substantial information regarding many aspects of Usher syndrome has accumulated since the cloning of the Usher type Ib gene 4 years ago and the discovery of the Usher type IIa gene in 1998. Although there are a number of excellent reviews on Usher type Ib, this is the first review on the molecular genetics of Usher type IIa [1, 14, 15].

Genetic heterogeneity of Usher syndrome

Usher syndrome exhibits significant genetic heterogeneity with at least nine independent loci having been identified and at least one more suspected (table 1). This is not a surprising fact given the clinical heterogeneity. The data to support this were published in the early 1990s in a series of papers in human genetics journals. The six loci for Usher syndrome type I (USH1a–f) are located on chromosomes 14q32, 11q13, 11p15, 10q, 21q21 and 10p [16–26]. The USH2 loci have been mapped to chromosome 1q41 (USH2A) and 5q11–13 (USH2B), respectively [27–32]. The USH3 gene is located on chromosome 3q [33–35]. While there are up to 10 different loci associated with the three clinical types of Usher syndrome, the majority of individuals diagnosed with USH1 exhibit linkage to 11q13 and can therefore be classified as USH1B patients. With regard to individuals who present an USH2 phenotype, the vast majority exhibit linkage to chromosome 1q41 and are therefore USH2A patients. While the remaining eight genes have yet to be identified, the USH1B and USH2A genes account for the majority of mutations in Usher patients.

The loci for several of the other subtypes have been refined, in some instances to several hundred kilobases of genomic DNA [36–38]. In addition, candidate genes have been assigned to certain Usher loci and are under investigation as possible Usher genes [39, 40]. Progress in the genetic and physical refinement of the various Usher loci as well as the isolation of promising candidate genes should result in the identification of additional Usher genes in the near future.

Usher syndrome type IB is the result of mutations in the unconventional MYO7A gene

The first to be identified was the USH1B gene located on chromosome 11q13.5 (fig. 1) [13]. The transcription unit of the USH1B gene occupies more than 100 kb of genomic DNA. Forty-eight of the 49 exons contain coding sequences [41]. Differentially spliced products are transcribed from the 48 exons. The largest, most abundant transcript is as large as 7.4 kb. Mutations were observed in the USH1B gene in five unrelated families segregating USH1 and exhibiting linkage to 11q13.5 [13]. The primary event leading to the consideration of MYO7A as a candidate for the USH1B gene was the observation that a candidate gene for a naturally occurring mouse deafness mutant known as *shaker-1* (*sh1*) was murine MYO7A [42]. The *sh1* mouse exhibits vestibular dysfunction, as well as neuroepithelial abnormalities and degeneration of the organ of Corti [43]. The gene responsible for the phenotype had been mapped to murine chromosome 7 in a region which is syntenic with human chromosome 11q13. Simultaneous with the discovery that the *sh1* phenotype was due to mutations in the murine MYO7A, it was also discovered that the USH1B gene was the human unconventional MYO7A. To date, several types of mutations have been described. The mutations include missense, nonsense, splice site, and insertions as well as small deletions (fig. 2).

Table 1. Classification of Usher syndrome by clinical and genetic type and chromosomal location.

Syndrome	Gene	Location	Retinitis pigmentosa	Hearing loss	Vestibular areflexia
Usher I	USH1A	14q32	prepubertal	profound	yes
	USH1B	11q13	prepubertal	profound	yes
	USH1C	11p14	prepubertal	profound	yes
	USH1D	10q	prepubertal	profound	yes
	USH1E	21q21	prepubertal	profound	yes
	USH1F	10p	prepubertal	profound	yes
Usher II	USH2A	1q41	teenage	sloping	no
	USH2B	5q11-13	teenage	sloping	no
	USH2C	unknown	teenage	sloping	no
Usher III	USH3	3q25	adult	progressive	variable

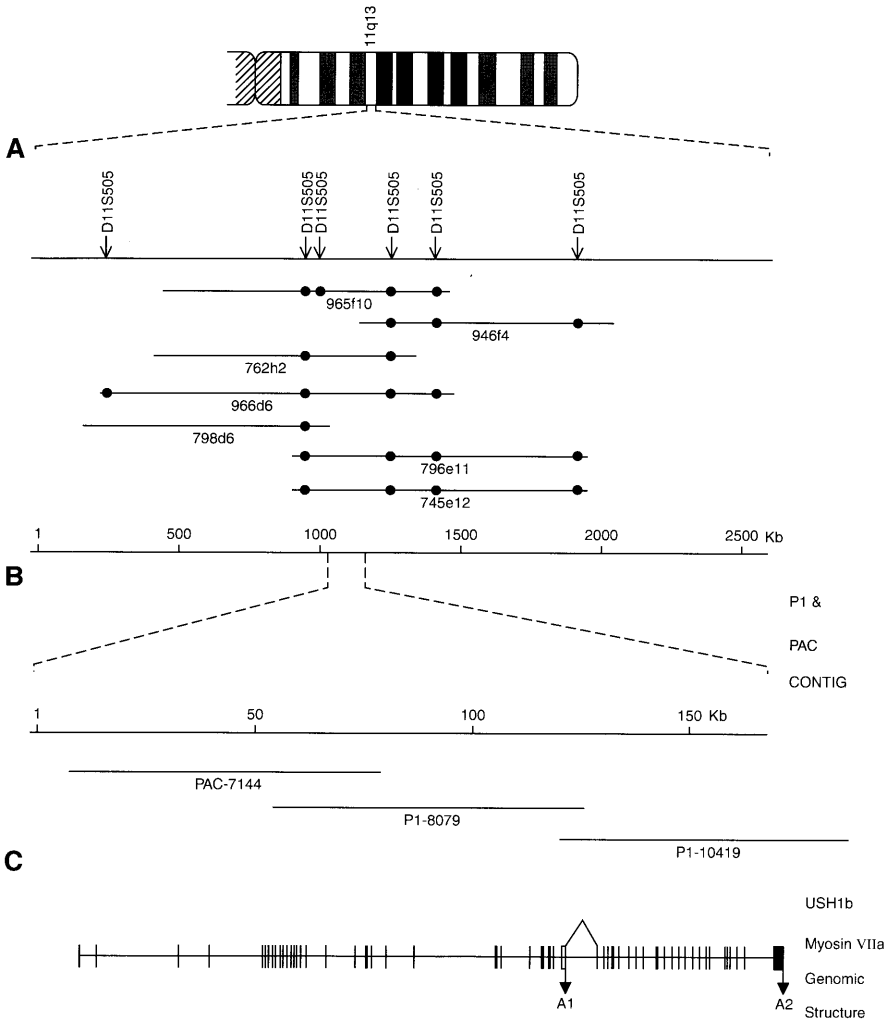

Figure 1. Physical map of the USH1B region. *Panel A* shows that the yeast artificial chromosome (YAC) contig of the 2.5 Mb region of human chromosome 11q13 containing the Usher type Ib gene. Some of the DNA markers used for generating the contig are shown. The YACs are shown as horizontal lines. The lengths of the YACs are drawn based on the available size information. The presence of a DNA marker on an individual YAC is indicated as a solid circle. *Panel B* shows P1 phage artificial chromosomes which carry genomic sequences corresponding to the gene of myosin VIIa. *Panel C* shows the exon/intron organization of the myosin VIIa gene. Vertical lines represent exons. Two splice variants (A1 and A2) are displayed.

MYO7A belongs to an evolutionarily conserved class of actin-based molecular motors known as the myosin superfamily [44]. Myosins interact with actin, and upon hydrolysis of adenosine triphosphate (ATP), move along the actin filaments due to the generation of mechanical force. There are at least 15 different classes of myosins based on the phylogenetic comparison of their conserved motor domains [45]. They are numbered according to their order of discovery. Multiple members exist within the different myosin classes, and their corresponding gene products are often expressed in the same cell [46]. The classes of myosins which are present in vertebrates are myosin-I, -II, -V, -VI, -VII, -IX, -X and -XV. The myosin about which most is known is myosin-II, the conventional filament-forming myosin involved in muscle contraction [47]. The other 14, referred to as unconventional myosins, are believed to perform fundamental functions in many actin-based cellular phenomena, including organelle trafficking, endocytosis, cell movement and signal transduction [44, 45]. In the last several years it has been learned that unconventional myosins play an important role in the physiology of the inner ear, and thus, in the physiology of hearing [14, 15, 49, 50].

The myosin superfamily members share a conserved structure consisting of three domains: an N-terminal head region which contains the actin and ATP binding sites; a neck region which contains the 'IQ' motifs

which interact with calmodulin and calmodulin-like proteins, and a class-specific tail region believed to be involved in subcellular localization, dimerization and protein-membrane interactions, thus conferring specificity of the different myosin isozymes (fig. 2). The MYO7A protein contains a 729-amino acid (aa) motor domain which has an ATP binding site (GESGAGKTE) located at aa position 158–166, and an actin binding site (FVRCIKPN) at aa position 622–629 [13, 42, 51–54] (fig. 2). Directly adjacent to the motor domain is the 129-aa neck domain, which contains five 'IQ' motifs arranged in tandem, each of which is comprised of approximately 23 aa residues. It has been predicted that repeats 1, 2 and 4 bind calmodulin and that repeats 3 and 5 bind calmodulin-like proteins. Following the neck region, MYO7A possesses a 1360-aa tail region which contains two repeats of approximately 460-aa residues located at positions 1016–1568 and 1708–2167, respectively. These repeats share 28% identity at the aa level, and both repeats contain a myosin tail homology domain (MyTH4 domain) as well as a domain known as the talin homology domain which is present in members of the band-4.1 superfamily of proteins [51].

The large 7.4-kb MYO7A transcript encodes the more abundant 250-kDa form of the protein. An additional transcript codes for a protein of 138 kDa which is identical with the 250-kDa form over the motor and neck domains but diverges at aa residue 1171 and continues for an additional 32 aa residues. Alternative splicing thus results in two forms of MYO7A which share the motor and neck domains but have different tails. Reverse-transcription polymerase chain reaction (RT-PCR) analysis detects transcripts of MYO7A in several human tissues including the retina, cochlea, liver, kidney and testis. In the mouse, transcripts are present in the retina, cochlea, liver and kidney [42]. Immunomicroscopy detects MYO7A protein in the cilia of a variety of cell types, and it has been suggested that MYO7A is a common component of cilia and microvilli [55].

MYO7A in the cochlea and retina

Insight into the cochlear and retinal abnormalities of the USH1B syndrome has been obtained by experiments utilizing both human and rodent systems. The spatial and temporal pattern of MYO7A expression in the developing and adult cochlea has been examined utilizing a variety of techniques. In situ analysis of MYO7A in human embryos identified transcripts in the developing otocyst in both the cochlea and vestibular neuroepithelia at 7–8 weeks of development [54]. In the guinea pig, indirect immunofluorescence using antibodies generated against the tail of MYO7A demonstrates the protein in the inner and outer hair cells of the sensory epithelium [52]. The hair cells of the cochlear neuroepithelia are actin-rich; indeed, five actin-rich subcellular domains have been described [48] to include the stereocilia, cuticular plate, adherens-junction network, circumferential actin belt and the lateral-wall cortical

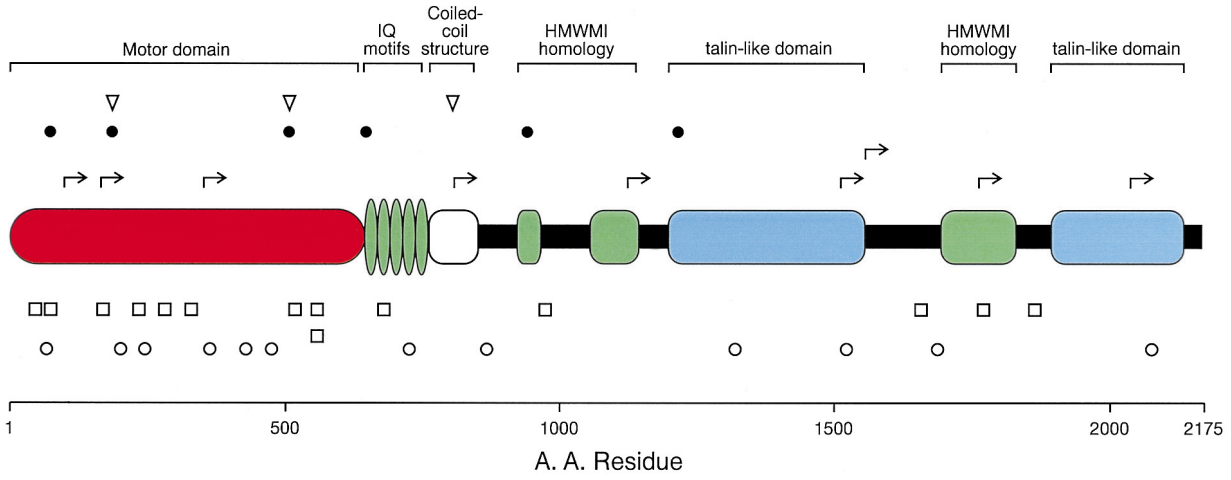

Figure 2. Schematic representation of the myosin-VIIa protein molecule showing the mutations found in the gene; nonsense (□), missense (○), frameshift ( ), splice site (●) and insertion/deletion in frame (▽).

cytoskeleton. The regions of the hair cells in which the MYO7A signal was most concentrated included the stereocilia and cuticular plate [52]. Intriguingly, MYO7A is also detected in the microtubule-enriched region located between the zona adherens and the cuticular plate of the hair cell known as the pericuticular necklace as well as in the cytoplasm [14]. MYO7A is not present in the supporting cells of the neuroepithelium or in other neural cells in the cochlea. Collectively, the data with respect to MYO7A expression support a role for MYO7A within the hair cells of the cochlea and vestibular apparatus.

In the last 2 years the role of nonconventional myosins in the process of hearing has become apparent [14, 15, 48–50]. Multiple myosin isozymes are present in the hair cells of the cochlea, and they appear to have important nonredundant functions, as evidenced by the different deafness mutants present in both humans and mice. For example, in addition to causing USH1B, mutations in the MYO7A gene also result in a recessive form of nonsyndromic deafness known as DFNB2 [56, 57]. In two separate studies, patients exhibiting linkage to the DFNB2 region were found to have mutations in the MYO7A gene. In addition, patients with atypical Usher syndrome with symptoms similar to an USH3 clinical phenotype also have MYO7A mutations [58]. These findings are interesting as they imply that the genetic background of the individuals with MYO7A mutations contributes to the severity of the disease. Mutations in the myosin-15 (MYO15) gene result in deafness in both mice and humans. The gene encoding human MYO15 is located on chromosome 17p11.2, and mutations in the gene account for the DFNB3 phenotype [59]. Murine MYO15 mutations result in the murine *shaker-2* (*sh2*) phenotype [60]. Murine myosin-VI (MYO6) mutations are responsible for the inner ear abnormalities in the deaf mouse mutant known as the *Snell's waltzer* mouse [61]. The different myosin gene mutations which result in deafness in both mice and men underscore the crucial role that myosins exert in the actin-rich environment of the cilia of the hair cells in the inner ear. The specific functions of the different myosins within the hair cells, however, have not yet been determined. Ultrastructural studies of the hair cells of *sh1* mice have revealed that the stereocilia bundles are disorganized [62]. This observation suggests that MYO7A is necessary for normal development of the stereocilia; however, the molecular events perturbed by defective MYO7A remain undefined.

The retinal defects of USH1B appear to be due to the lack of MYO7A within the retinal pigment epithelium (RPE) and photoreceptor cell layers of the retina. A study of MYO7A expression in the developing human retina revealed expression of MYO7A in the RPE at 5, 7 and 8 weeks of development [54]. During this early stage of development no transcripts were detected in the developing neural retina. By 19 weeks, MYO7A expression was apparent in the neural retina, confined to the photoreceptor layer. At adulthood, the MYO7A protein was detected specifically within the rod and cone cells [63]. Immunogold labeling detected MYO7A in the connecting cilia of both rods and cones. Interestingly, the highest concentration of MYO7A was in the connecting cilia and surrounded the microtubule doublets near the plasma membrane [64]. In the rodent systems analyzed, MYO7A expression is confined to the RPE and is not detectable in the neural retina [52, 54, 63]. This observation may explain the lack of retinal degeneration in the *sh1* mouse. These mice exhibit an abnormal distribution of melanosomes in the RPE cells, implying a role for MYO7A in organelle transport in the RPE; this does not, however, account for the photoreceptor dysfunction observed in USH1B [65].

In conclusion, although the specific cell types in the retina and cochlea with respect to the expression of MYO7A are known, the molecular function of MYO7A in these cells remains unknown. Several different actin-mediated processes of hair cells and photoreceptors including phagocytosis of outer disk segments by the RPE cells, organelle transport and neurotransmitter release have been postulated as potential pathways which could be disrupted by a defective or absent MYO7A protein. Whether or not one or more of these processes is involved in USH1B remains to be determined.

USH2A is the result of mutations in a putative extracellular matrix molecule

The second Usher gene to be identified was the USH2A gene at 1q41 by positional cloning (fig. 3) [12]. Although it was first believed that the USH1 phenotype was the most common of the Usher phenotypes, recent studies suggest that the USH2 phenotype is as prevalent if not more so than the USH1 phenotype [4]. Mutations in the USH2A gene may account for over half of all Usher cases. Three different mutations were found in a complementary DNA (cDNA) encoding a protein of 171 kDa which contains 10 laminin epidermal growth factor (LE) motifs followed by 4 fibronectin type III (F3) motifs, motifs most often observed in proteins comprising the extracellular matrix and in cell adhesion molecules (fig. 4) [66]. The mutations were found exclusively in USH2A patients exhibiting linkage to 1q41 and all mutations segregated with the clinical phenotype, demonstrating conclusively that this was the USH2A gene.

The USH2a cDNA is 6330 nucleotides in length, with a large open reading frame (ORF) extending from nucle-

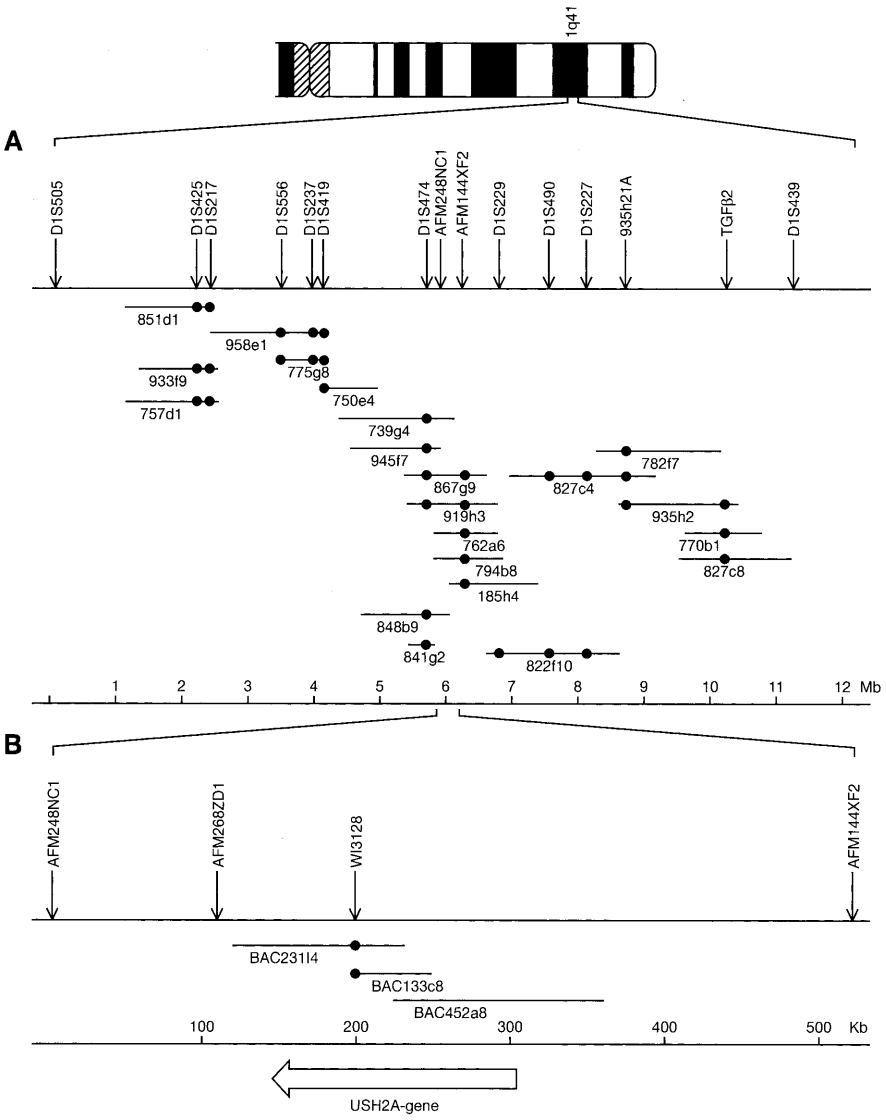

Figure 3. Physical map of the USH2A region. *Panel A* displays the YAC contig of the 12 Mb region of human chromosome 1q41. YAC DNA markers are shown as in figure 1. *Panel B* shows bacterial artificial chromosomes that contain the USH2A gene.

otide position 371 to 5023. Conceptual translation of the ORF predicts a protein containing 1551 aa residues with an isoelectric point of 7.45. The USH2A protein shares 32% identity and 47% similarity between residues 300 and 1050 with all laminin family members [66]. In the region of the USH2A protein that exhibits similarity to the laminin family, the protein contains 10 LE repeats, each with approximately 50 aa residues arranged in tandem (fig. 4). Members of the laminin superfamily of proteins are one of the most common components of the basement membranes of all tissues, and the LE motif is common to other extracellular matrix proteins as well. Due to the high sequence similarity between the USH2A protein and the laminins, one might expect that the USH2A protein is a novel form of laminin. This is unlikely, as the homology between the USH2A and the laminin proteins ends at position 1050; furthermore, the USH2A protein lacks the characteristic coiled-coil domains of laminins in the carboxy-terminal region. From aa residue 1090 to 1500, the USH2A protein contains another motif common to extracellular matrix proteins and cell adhesion molecules: the F3 domain. Between residues 1090 and 1500, the USH2A protein contains four F3 repeats of approximately 100 residues each, arranged in tandem. Throughout the F3 motif region, the USH2A protein exhibits similarity to a wide variety

of proteins containing F3 domains, most of which are found in the extracellular matrix. The greatest homology, however, is to neogenin and to the leukocyte common antigen-related proteins, both of which contain transmembrane domains and function as cell-adhesion molecules [67, 68]. The USH2A protein contains two potential transmembrane helices at position 1366–1383 (outside to inside helix) and 1447–1465 (inside to outside helix). Although the USH2A protein contains two potential transmembrane domains, it is important to emphasize the hypothetical nature of the transmembrane domains, as the numerical threshold for statistical significance utilizing the Transmembrane Prediction Program (TMpred) was just reached [69]. Whether the USH2A protein actually spans the membrane must be experimentally determined before one can state whether it represents a novel extracellular matrix protein or a novel cell-adhesion molecule. The N-terminal 20 residues of the USH2A protein exhibit characteristics of a signal peptide and may represent a signal for secretion. Consistent with the homology data, which suggest that the USH2A protein is secreted into the extracellular matrix, the USH2A protein also contains 18 potential N-glycosylation sites dispersed throughout the length of the predicted protein.

The USH2A gene expression pattern is virtually confined to the retina and cochlea. Northern blot analysis of a variety of human tissues utilizing the USH2A cDNA as a probe detected transcripts of approximately 6.5, 5.0 and 1.9 kb in the adult human retina but in no other tissues. RT-PCR detected USH2A transcripts in human embryonic total eye and cochlea RNA preparations. Weak signals were also detected in human brain and kidney; however, due to the sensitivity of RT-PCR, it is not known whether the message was present at physiologically relevant levels. Collectively, the Northern blot and RT-PCR data suggest that the USH2A gene is highly tissue-specific with respect to expression pattern.

Experiments which define the specific cell or cells which produce the USH2A protein (i.e. immunohistochemistry and RNA in situ analysis) are at present being performed. As the USH2A-predicted protein has the sequence homology and characteristics of a protein excreted into the extracellular matrix, one can generate hypotheses with respect to its function in the cochlea and retina. There is, in fact, scientific precedence which demonstrates the importance of extracellular matrix molecules in the development and physiology of the inner ear. Mutations in the α-tectorin gene, which encodes a protein located in the specialized extracellular matrix of the tectorial membrane, result in a form of deafness [70]. Both autosomal recessive and X-linked Alport syndrome, a syndrome characterized by progressive glomerulonephritis and deafness, are due to mutations in several different collagen type-IV genes [71, 72]. It is plausible that the USH2A protein is a critical component of either the membranous labyrinth of the cochlea or the tectorial membrane. In the retina, the specialized extracellular matrix which surrounds the photoreceptors and the Muller cell processes is known as the interphotoreceptor cell matrix (IPM). The IPM contains both soluble and insoluble fractions, is particularly rich in glycoproteins and performs functions crucial to the normal physiology of the neural retina [73]. These functions include trophic support, retinol transport and retina attachment. The IPM differs in composition from other extracellular matrices in that it does not contain fibronectin or the high molecular weight laminins. The IPM does, however, contain s-laminin, which is a form of the laminin B1 subunit [74]. It has been proposed that this form of laminin contributes to the formation of rods during the development of the retina [75]. As the USH2A protein is most likely an extracellular matrix component and as most retinitis pigmentosa mutations are found in genes which encode proteins present in rods and cones, a reasonable hypothesis is that the USH2A protein is a component of

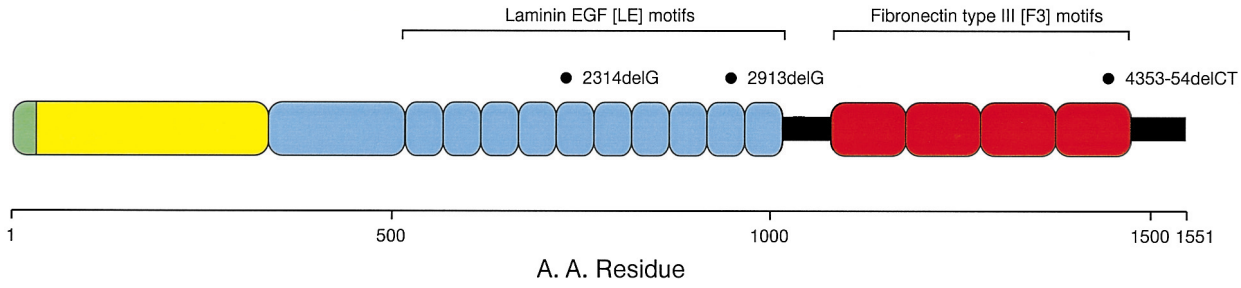

Figure 4. Schematic representation of the USH2A protein molecule showing the position of the mutations found in the gene.

the IPM. In the final analysis, the elucidation of the function of the USH2A protein in the cochlea and retina is dependent upon precise localization of the protein, as well as other experiments.

Summary and conclusions

Of the 10 Usher syndrome genes, two have been identified: USH1B and USH2A. An interesting question arises regarding the USH1B gene, which encodes MYO7A, and the USH2A gene, which encodes a putative extracellular matrix molecule. What, if any, relation is there between these two different classes of molecules? To address this question, one might examine the respective causes of RP and sensorineural hearing loss. RP is the end result of a mutation in a wide variety of genes [76]. Whereas the classical RP genes encode proteins which are located in the photoreceptor cells and are components of the phototransduction pathway, recent identification of other RP genes illustrates that this is not always the case. For example, mutations in peripherin, which is believed to be a structural component important in anchoring the outer disk segments of the photoreceptors to the cytoskeleton, result in RP [77]. Other RP genes include RPE65, TULP1 and ABCR [78–81]. It has been suggested that the common thread between these RP genes is ultimately the induction of the apoptotic pathway, leading to degeneration of the retinal cells [76]. Regarding sensorineural hearing loss, it is predicted that up to 100 loci are responsible for nonsyndromic and syndromic deafness [82]. Mutations resulting in nonsyndromic deafness have been detected in a variety of genes which encode different types of proteins. The most common gene defect observed in the deaf population stems from mutations in the gap-junction protein known as connexin 26 [83]. Other deafness genes include α-tectorin, diaphanous, a POU domain containing transcription factor as well as three different unconventional myosin genes [70, 84, 85].

Given the wide variety of gene mutations which result in either RP or deafness, one is led to speculate that although the Usher syndromes overlap clinically, the molecular pathways may be separate and distinct. With the recent discovery of the USH2A gene, one posits whether the respective Usher syndromes will be shown to exhibit molecular pathological heterogeneity as well. With regard to this issue, other genes are currently under investigation as possible Usher syndrome genes. These include a human homologue (SIAH-2) of the *Drosophila* seven-in-absentia gene (sina) as a candidate for the USH3 gene, and HuEMAP, which is a human homologue of the echinoderm microtubule-associated protein (EMAP) and is a candidate for the USH1a gene [39, 40]. SIAH-2 is an intriguing candidate, as the sina gene performs an important role in photoreceptor development in *Drosophila* [86]. It is believed that SIAH-2 is involved in the ubiquitin-proteosome pathway and thus is involved in protein degradation [87]. HuEMAP is a candidate for the USH1A gene, as it maps to the USH1A locus and is likely a novel type of microtubule-associated protein. As the USH1B gene, (MYO7A) is a component of the cytoskeleton, a microtubule-associated protein such as HuEMAP would be consistent with a cytoskeletal abnormality resulting in USH1. In conclusion, the issue of molecular pathological heterogeneity as well as others will be addressed when additional Usher genes and the functions of their corresponding protein products are elucidated.

Acknowledgments. Janos Sumegi is supported by a grant from NIH-NIDCD 5P01 DCO1813-15 and by the Nebraska Research Initiative Fund. We thank Dr. Thomas A. Seemayer for the critical review of the manuscript, J. Edwards for the artwork and Tina Winekauf for the preparation of the manuscript.

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