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Review Article

The molecular genetics of Usher syndrome

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Association of sensorineural deafness and progressive retinitis pigmentosa with and without a vestibular abnormality is the hallmark of Usher syndrome and involves at least 12 loci among three different clinical subtypes. Genes identified for the more commonly inherited loci are *USH2A* (encoding usherin), *MYO7A* (encoding myosin VIIa), *CDH23* (encoding cadherin 23), *PCDH15* (encoding protocadherin 15), *USH1C* (encoding harmonin), *USH3A* (encoding clarin 1), and *USH1G* (encoding SANS). Transcripts from all these genes are found in many tissues/cell types other than the inner ear and retina, but all are uniquely critical for retinal and cochlear cell function. Many of these protein products have been demonstrated to have direct interactions with each other and perform an essential role in stereocilia homeostasis.

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There are many syndromes that affect both the visual and auditory systems but Usher syndrome is the most common genetic cause of deafness and blindness among school age children (1–3). Usher syndrome was first described in 1858 (but not by name) when the German ophthalmologist Alfred von Graefe published an article describing a family in which deafness and blindness cosegregated (4). Kloepfer and Laguaite provide an excellent review of the early literature regarding Usher syndrome (5). Its prevalence is currently estimated in developed countries at 3.8–4.4 per 100,000 live births (6,7) and is found much more commonly among those with retinitis pigmentosa than among those that are deaf (8, 9). Hearing loss is more prevalent than retinitis pigmentosa (estimated at 12.5–27.0 per 100,000 live births) in developed countries (10, 11). The prevalence of deafness among children in a developed country was recently estimated from a survey of deaf children in the United Kingdom (population 59 million) at 1.07 cases/1000 for 3 year olds to 2.05 for those aged 9–16 (12). Of those reported to be deaf, 5.7% also had problems in the visual system out of a total of 27.4% describing traits in addition to hearing loss (12, 13). These numbers are in agreement with surveys performed in the United States where it was found that at least 50% of those with hearing problems were genetic in origin (14).

The majority of patients with Usher syndrome usually fall into one of these clinically distinct categories. Usher syndrome type I (USH1) patients have profound hearing loss and vestibular dysfunction from birth. In addition, night blindness appears earlier in life than USH2 patients, who tend to have less severe hearing loss and normal vestibular function (15–17). A third classification (USH3) is found most frequently among Finnish patients where the hearing loss and retinitis pigmentosa are progressive, with variable vestibular dysfunction (18). Some patients affected with Usher syndrome cannot be easily categorized into one of three subtypes, these show an atypical clinical designation (19).

Usher syndrome is heterogeneous, which means that different mutated genes can cause the same phenotype. In Table 1 we list all known loci including those for which there are mouse models or genes cloned along with information on the

Table 1. Molecular classification of Usher syndrome

Usher locus	Chomosomal location	Overlapping non-syndromic locus	Gene	Protein	Mouse model	Relative abundance in a sampling of mostly US Usher patients	Relative abundance in a sampling of Pakistani deaf children
USH type I						Relative to USH1	Relative to USH1
A(95)	14q32	–	–	–			
B (22)	11q13.5	DFNB2 DFNA11 (48, 51, 97)	MYO7A	Myosin VIIa	Shaker 1 (56)	49% (50)	42.9%
C (23)	11p15.1	DFNB18 (44)	USH1C	Harmonin		>2% (50)	12.2%
D (26)	10q22.1	DFNB12 (43)	CDH23	Cadherin 23	Waltzer (74)	33% (combined D&F, 50)	28.6%
E (96)	21q21	–	–	–			
F (27)	10q21.1	DFNB23 (42)?	PCDH15	Protocadherin 15	Ames waltzer (79)	33% (combined F&D, 50)	12.2%
G (28)	17q25.1	–	SANS	SANS	Jackson shaker (82)		
?	Unknown					16% (50)	4.1%
USH type II						Relative to USH2	
A (20)	1q41	Non-syndromic RP(46)	USH2A	Usherin		>70% (85)	
B (29)	3p23-24.2	DFNB6 (45)?	–	–			
C (110)	5q14.3-21.3	–	–	–			
? (85)	Unknown	–	–	–			
USH type III							
A (18)	3q25.1	–	USH3A	Clarin 1			
B (64)	20q	–	–	–			

relative abundance of some of these loci. It is known from many years of research performed at the Boys Town National Research Hospital (Omaha, NE) that markers at 1q41 (*USH2A*) cosegregate with the phenotype in many USH2 families (20, 21) and markers at 11q13.5 (*USH1B*) cosegregate with the phenotype in many USH1 families (22). Other groups found that samples from USH1 families with an Acadian ancestry cosegregate with a locus at 11p15.1 (*USH1C*) (23, 24). It was then found that markers at 3q25 cosegregate with the phenotype in Finnish families inheriting USH3 (18). Families supporting sufficient statistical power to either refine known loci or to define new linkage locations have aided in the identification of several loci (25). *USH1D* was localized to chromosome 10q22.1 (26), *USH1F* was localized to 10q21.1 (27), *USH1G* was localized to 17q25.1 (28), and *USH2B* was localized to 3p23-24.2 (29).

Linkage data from over 400 Pakistani families is in agreement with these types of measurements assayed in other parts of the world. Approximately 10% of families enrolled in schools for deaf children have USH1. In relative abundance, almost half are linked to the *USH1B* locus followed by approximately 30% linked to *USH1D* and the remainder equally divided between *USH1C* and *USH1F* (unpublished data, SR, SR and ZMA, see Table 1).

Mutations in seven different genes are currently ascribed as the cause of Usher syndrome: *USH1B* encoding myosin VIIa (30), *USH1C* encoding harmonin (31, 32), *USH1D* encoding cadherin 23 (33, 34), *USH1F* encoding protocadherin 15 (35, 36), *USH1G* encoding SANS (37), *USH2A* encoding usherin (38), and *USH3A* encoding clarin-1 (39–41). These genes were identified through the discovery of a mouse homolog, or by positional cloning. Many of the Usher loci also overlap the same chromosomal location as loci responsible for non-syndromic deafness. Recessive non-syndromic deafness locus 12 (*DFNB12*) (42) mapped to the same region of chromosome 10 as *USH1D* (26, 43), *DFNB18* to *USH1C* (23, 44), *DFNB23* to *USH1F* (27, 42) and *USH2B* to *DFNB6* (29, 45).

With many of the Usher syndrome genes now identified, a more careful examination of the phenotype associated with mutations in any one gene is possible, providing some interesting observations. One mutation in the *USH2A* gene, Cys759Phe, accounts for at least 4.5% of recessive retinitis pigmentosa without hearing loss, and represents the single largest cause of non-syndromic blindness discovered to date among a sampling of 224 mostly unrelated North Americans (46). Likewise, different mutations in the gene responsible for *USH1D* can be found to cause a phenotype similar to USH1, USH2, USH3 and recessive non-syndromic deafness (34, 47). Moreover, mutations in the gene responsible for *USH1B* are shown to cause USH1 or USH3-like symptoms, as well as dominant non-syndromic deafness (48, 49). We conclude from these studies that there are limitations to the current clinical classification of families inheriting Usher syndrome.

We can conclude from these clinical applications of molecular genetics to the study of Usher syndrome that many of the disease-causing genes are now identified, and that the more abundant mutant alleles are known. Much more study is needed to understand the pathophysiology of these genes so that at some future date therapy will be possible.

Cloned Usher loci and mouse models

USH1B/DFNB2/DFNA11 and *shaker 1*

USH1B was first localized among families from the United States enrolled for study at the Boys Town National Research Hospital (22, 23) and is the most common cause of USH1 in the United States (50) and Pakistan (unpublished data, SR, SR and ZMA). Subsequent to the initial mapping studies identifying *USH1B*, a large consanguineous Tunisian family segregating non-syndromic recessive deafness mapped to 11q14, was assigned the locus symbol *DFNB2* and hypothesized to be due to allelic mutations of the gene for *USH1B* (51). Recently, a detailed ophthalmological examination of affected individuals from the original *DFNB2* Tunisian family showed early signs of retinitis pigmentosa, suggesting that this family probably has an atypical Usher syndrome (52, 53). Moreover, a gene for dominantly inherited non-syndromic deafness segregating in a Japanese family was also mapped within the linkage region of *USH1B*/*DFNB2* (54) and is an allelic variant responsible for dominant non-syndromic locus 11 (*DFNA11*).

Homozygous shaker 1 (*sh1*) mice are deaf, and the defective gene maps to chromosome 7 (55), a region showing conserved synteny with homologs on human chromosome 11 harboring the gene for *USH1B*/*DFNB2*/*DFNA11* (22, 51, 54). The identification of unconventional myosin VIIa as the gene mutated in *sh1* (56) led quickly to the cloning of the human ortholog. As suspected, different mutant alleles of *MYO7A* cause *USH1B*, recessive, non-syndromic deafness *DFNB2* and dominant non-syndromic deafness *DFNA11* (30,

48, 57, 58). This later mutation is uniquely found in the coiled–coil domain responsible for dimerization of *MYO7A*, resulting in a dominant negative effect (48). A summary of *MYO7A* mutations identified in *USH1B, DFNB2*, and *DFNA11* subjects is shown in Table 2. These mutations are distributed across nearly all exons of *MYO7A*, but most are located in exons encoding the motor domain (59, 60). Unconventional myosins interact with actin through their motor domain and upon hydrolysis of ATP exert force to travel along actin filaments (61). *MYO7A* has 49 coding exons (62) and spans approximately 87 kb of genomic DNA on chromosome 11q14.1. The predicted human protein encoded by *MYO7A* is 2215 amino acids (Fig. 1) (63), and has 96% identity and 98% amino acid similarity with the mouse orthologue. A splice isoform, *MYO7b*, encoding only 1203 amino acids was also reported, but no functional studies on this shortened *MYO7A* have been reported (63). Additional details on the functional aspects of this protein are available elsewhere (64, 65).

USH1C/DFNB18

USH1C was mapped to chromosome 11p15.2-p14 (23) and subsequently refined to a 3-cM interval (24). Two groups reported the positional cloning of the *USH1C* gene encoding harmonin (31, 32). The structure of USH1C is complex with many alternatively spliced isoforms from 20 primary and eight alternative exons (32, 66).

Recently, we and others reported allelic variants of *USH1C* causing non-syndromic recessive deafness, designated *DFNB18* (44, 67, 68). Interestingly, the mutation 238-239insC, which was previously reported in six unrelated subjects of European or Pakistani ancestry inheriting *USH1C*, was found in two additional consanguineous Pakistani families and may be a common cause of *USH1C* in Pakistan (see Table 2) (67).

Harmonin has 94% identity and 95% amino acid similarity with mouse *Ush1C* isoform a1. In humans, harmonin has many tissue-specific isoforms (66). In the mouse inner ear, there are at least eight different alternatively spliced variants of harmonin (32). The predicted isoforms have two or three PDZ domains, one or two coiled–coil regions and, in some splice variants, a PST (proline, serine, threonine) domain (32). The harmonin b isoforms are found only in the inner ear, whereas the other isoforms have broader expression patterns (32). In the inner ear, harmonin is expressed in the organ of Corti as well as in the five sensory areas of the vestibule (32). In the cochlea, harmonin isoform b expression is detected by E15 in differentiating hair cells at the basal turn of the cochlea and expression at the tips of the stereocilia can be seen as late as P30 (69). After P30, harmonin isoform b expression was undetectable, but the proteins encoded by other harmonin isoforms are found expressed in the cuticular plate and stereocilia throughout the post-natal life of the mice (69). A similar expression pattern is observed for vestibular hair cells (69).

PDZ domains are motifs that interact in a sequence-specific fashion with the C-terminal peptides or internal peptides that fold into a β-finger (see Fig. 1) (70). Typically, PDZ domains are 80–90 amino acids long and show some sequence divergence, which probably reflects diversity in binding specificity (70). The most general function of PDZ domains may be to localize specific ligands to the appropriate plasma membrane domain (71, 72).

Recently, two groups independently reported the interaction between harmonin PDZ domains, the second FERM and MyTh4 domains of myosin VIIa, and the cytoplasmic domain of cadherin 23 (69, 73). The first PDZ domain of harmonin interacts with myosin VIIa, while the second PDZ domain binds the cytoplasmic region of cadherin 23, and the third PDZ domain along with the PST domain might interact with F-actin. The interaction of all three proteins forms a transmembrane complex essential for the organization and stabilization of stereocilia (69, 73). The resulting complex along with cadherin 23 is found near the tip of the stereocilia, inferred from the misdirected transport of harmonin in myosin VIIa mutant mice (69).

USH1D/DFNB12 and *Waltzer (v)*

USH1D and *USH1F* map to human chromosome 10q in an interval that includes two non-syndromic recessive deafness loci, *DFNB12* and *DFNB23* (26, 27, 42, 43). The region of conserved synteny in the mouse includes three deafness mutants, *Waltzer* (*v*), the modifier locus (*mdfw*) of deaf waddler (*dfw*) and the gene for age-related hearing loss (*Ah1*) (74–76). *Waltzer* (*v*) mice exhibit profound deafness with head tossing reflecting vestibular dysfunction. Ultrastructural analysis of the inner ears of *v* homozygous mice show normal inner and outer hair cell formation but the stereocilia are abnormal in number, organization, shape and position relative to the kinocilium (74).

Table 2. A summary of the published mutations causing Usher syndrome

Gene	Locus	Mutation	Domain	References
MYO7A	*DFNA11*	Δ886-888	Coiled coil	(48)
	DFNB2	R244P	Motor domain	(57)
		M599I	Motor domain	(58)
		IVS3-2	Motor domain	(57)
		IVS24-21	FERM domain	(98)
		1199insT	MyTH4	(57)
	USH1B	L16X	Motor domain	(98)
		G25R	Motor domain	(97)
		A26E	Motor domain	(99)
		C31X	Motor domain	(60)
		V67M	Motor domain	(99)
		75delG	Motor domain	(60)
		R90P	Motor domain	(99)
		120delC	Motor domain	(60)
		I134N	Motor domain	(99)
		R150X	Motor domain	(30)
		IVS5 + 1	Motor domain	(62)
		R212C	Motor domain	(30)
		R212H	Motor domain	(30)
		G214R	Motor domain	(62)
		ΔD218-I219	Motor domain	(30)
		A226T	Motor domain	(100)
		Q234X	Motor domain	(30)
		R241S	Motor domain	(98)
		R244P	Motor domain	(57)
		269delAAG	Motor domain	(99)
		R302H	Motor domain	(60)
		E314X	Motor domain	(60)
		Y333X	Motor domain	(60)
		A397D	Motor domain	(62)
		E450Q	Motor domain	(60)
		A457V	Motor domain	(99)
		468 + Q	Motor domain	(60)
		P503L	Motor domain	(60)
		G519D	Motor domain	(99)
		IVS13-1	Motor domain	(99)
		IVS13-8	Motor domain	(60)
		521delC	Motor domain	(97)
		532delA	Motor domain	(60)
		542insC	Motor domain	(99)
		C628X	Motor domain	(101)
		R634X	Motor domain	(101)
		R666X	Motor domain	(98)
		R669X	Motor domain	(101)
		724delC	Motor domain	(101)
		IVS18+1	Motor domain	(62)
		A826T	IQ motif	(62)
		G955S	Post coiled–coil domain	(102)
		E960X	Post-coiled–coil domain	(98)
		E968D	Post-coiled–coil domain	(99)
		E1080X	MyTH4 domain	(103)
		E1170K	MyTH4 domain	(103)
		IVS28 + 2	MyTH4 domain	(102)
		R1240E	MyTH4 domain	(98)
		R1240Q	MyTH4 domain	(99)
		IVS29 + 2	Post-MyTH4 domain	(101)
		A1288P	FERM domain	(98)
		R1343S	FERM domain	(98)
		1346delTTC	FERM domain	(99)
		R1602Q	FERM domain	(101)
		A1628S	SH3 domain	(101)
		Y1719C	Post-SH3 domain	(98)
		R1743W	MyTH4 domain	(99)
		Q1798X	MyTH4 domain	(98)
		L1858P	Post-MyTH4 domain	(99)
		R1861X	Post-MyTH4 domain	(62)

Table 2. Continued

		IVS40-2	Pre-FERM domain	(99)
		P1887L	FERM domain	(99)
		2018delG	FERM domain	(99)
		2065delC	FERM domain	(62)
		2119-2215del2 kb	FERM domain	(62)
		G2137E	FERM domain	(102)
		G2163S	FERM domain	(98)
		G2167D	FERM domain	(101)
	Atypical *USH1B*	L651P cpd. het. w/R1602Q	Motor domain and FERM domain	(49)
USH1C	*USH1C*	IVS1 + 1G > T	Amino terminus	(104)
		R31X	Amino terminus	(104)
		216G > A	Amino terminus	(31)
		238-239insC	Amino terminus	(31, 32, 104)
		IVS5 + 1G > A	PDZ1	(104)
		IVS5-2delA	PDZ1	(32)
		IVS8 + 2T > G	PDZ2	(67)
		769-770ins36	PDZ2	(67)
		G431V	Coiled coil	(68)
	DFNB18	P608R	PST	(68)
		R620L	PST	(68)
		R636C	PST	(68)
		IVS12 + 5G > C	PDZ2	(67)
CDH23	*DFNB12*	D124G	CA1	(47)
		N452S	CA4	(47)
		L480Q	CA5	(47)
		R582Q	CA6	(47)
		D990N	CA9	(34)
		R1060W	CA10	(47)
		G1186D	CA11	(47)
		D1341N	CA13	(105)
		A1586P	CA15	(47)
		E1595K	CA15	(47)
		D1846N	CA17	(47)
		D2045N	CA19	(34)
		D2148N	CA20	(47)
		D2202N	CA21	(34)
		R2465W	CA23	(47)
		R2608H	CA24	(47)
		I2950N	CA27	(34)
		R2956C	CA27	(34)
		P3059T	CA27-TM	(34)
	USH1D	Q58X	CA1	(47)
		1087delG	CA4	(47)
		A484P	CA5	(47)
		F995L	CA9	(47)
		P1206R	CA11	(47)
		3840insATGA	CA12	(47)
		Q1294X	CA12_	(34)
		Q1496H	CA14	(33)
		T1904T	CA18	(106)
		IVS45 + 1	CA19	(34)
		R2107X	CA20	(34)
		6933delT	CA21	(106)
		IVS51 + 5	CA23	(33)
		9626insC	CYTO	(47)
		T1209A	CA11/CA12_	(47)
		E2103X	CA20	(47)
		S2517G	CA23/CA24_	(47)
		E2624E	CA25	(47)
		R3175H	CYTO	(47)
	Atypical *USH1D*	193delC	CA1	(47)
		IVS4 + 1	CA1	(47)
		1112delT	CA4	(47)
		IVS20 + 1	CA7	(47)
		T1035T	CA10	(47)
		delM1281	CA12	(33)

Table 2. Continued

		R1502X	CA14	(47)
		R1746Q	CA16/CA17	(33)
		IVS45-9	CA19	(106)
		6155delC	CA19	(47)
		6968delC	CA22	(47)
		G2744S	CA26	(47)
		R2833G	CA26	(47)
		IVS66 + 1	CYTO	(34)
PCDH15	*USH1F*	R3X	Signal peptide	(35, 36)
		R245X	CA2	(111)
		1471delT	CA3	(36)
		IVS27-2A > G	CA11	(35)
SANS	*USH1G*	L48P	Ankyrin repeat 1	(37)
		186-187delCA	Ankyrin repeat 1	(37)
		393insG	Post 3rd Ankyrin repeat	(37)
		829-848del	Post 3rd Ankyrin repeat	(37)
USH2A	*USH2A*	R34X	Pre-laminin type VI	(107)
		R63X	Pre-laminin type VI	(107)
		238-239insCGTA	Pre-laminin type VI	(108)
		C163Y	Pre-laminin type VI	(107)
		V218E	Pre-laminin type VI	(109)
		V230M	Pre-laminin type VI	(107)
		L260X	Pre-laminin type VI	(85)
		921-922insCAGC	Laminin type VI domain	(85)
		C319Y	Laminin type VI domain	(85)
		R334W	Laminin type VI domain	(108)
		N346H	Laminin type VI domain	(85)
		W409X	Laminin type VI domain	(85)
		C419F	Laminin type VI domain	(85)
		C536R	1st laminin EGF-like	(107)
		L555V	1st laminin EGF-like	(109)
		1679delC	1st laminin EGF-like	(85)
		Q566X	1st laminin EGF-like	(85)
		R626X	2nd laminin EGF-like	(85)
		1965delT	3rd laminin EGF-like	(107)
		Q675X	3rd laminin EGF-like	(107)
		Q684Q	3rd laminin EGF-like	(85)
		G713R	4th laminin EGF-like	(107)
		2299delG	5th laminin EGF-like	(38)
		C847X	7th laminin EGF-like	(85)
		Q933X	8th laminin EGF-like	(107)
		2878delAA	9th laminin EGF-like	(107)
		2898delG	9th laminin EGF-like	(38)
		3840-41GA→CT	3rd Fibronectin type III	(85)
		R1295X	3rd Fibronectin type III	(85)
		4338-9delCT	4th Fibronectin type III	(38)
		4510-4511insA	Carboxy tail region	(85)
		T1515M	Carboxy tail region	(108)
	RP without HL	C759F	5th laminin EGF-like	(46)
USH3A	*USH3A*	N48K	Pre-1st TM	(40, 41)
		149delCAGG/ insTGTC-CAAT	Pre-1st TM	(41)
		165delC	Pre-1st TM	(41)
		187-209del23bp	Pre-1st TM	(40)
		Y63X	Pre-1st TM	(40)
		M120K	1st TM	(39, 41)
		L150P	2nd TM	(41)
		459-461delATT	2nd TM	(39, 41)
		Y100X	2nd TM	(39, 41)

Atypical cases of Usher syndrome are to draw attention to mutations that do not fit the typical Usher phenotype associated with mutations found in a particular gene. In some instances, it is used synonymously with the USH3 phenotype. The following abbreviations are used in this table: FERM: a homology shared among the 4.1, ezrin, radixin and moesin proteins; MyTH4: a myosin tail homology 4 domain found among myosins and kinesins; IQ motif: calmodulin-binding motif containing isoleucine and glutamine residues; SH3: first described in the Src tyrosine kinase, a domain involved in binding to other proteins through proline and hydrophobic amino acids; PDZ: a protein domain first identified by homologies in PSD-95, Dlg and Z0-1/2, that can bind to specific sequences in other proteins; CA: a domain found among cadherins, these extracellular repeats are involved in Ca^{2+} mediated cell–cell adhesion; CYTO: refers to a cytoplasmic region of a transmembrane protein; TM: refers to the transmembrane domain of a protein; RP: refers to retinitis pigmentosa; HL: refers to hearing loss. Please see http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml for additional domain information regarding these and other protein domains.

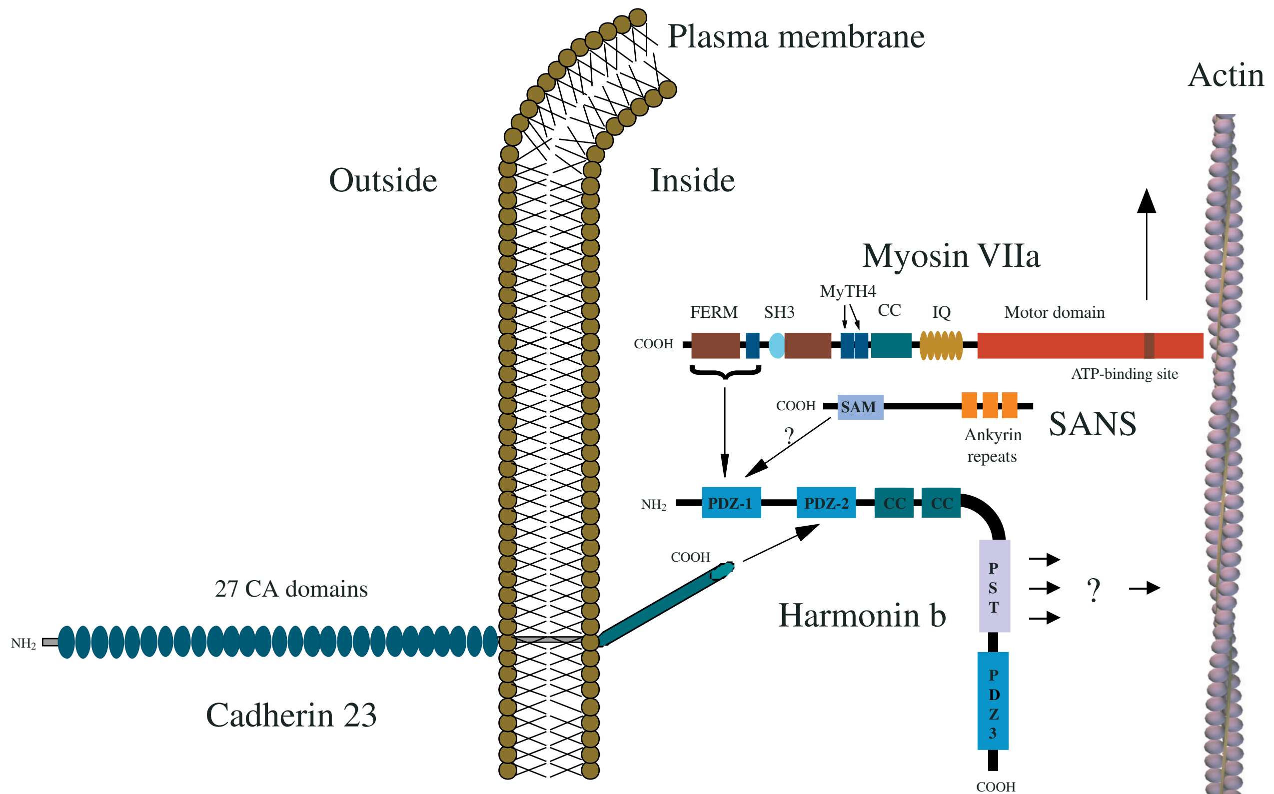

Fig. 1. Schematic representation of the predicted structure of Myosin VIIa with a motor head region which contains binding sites for ATP and actin followed by a neck region containing five isoleucine-glutamine (IQ) motifs (yellow). The tail region consists of one coiled–coil region (CC), two myosin tail homology four domains (MyTH4), one SH3 domain and two FERM domains. Also shown is a schematic representation of the predicted structure of harmonin isoform b with three PDZ domains, a CC domain (for self-multimerization) and a PST region (proline, serine and threonine rich region that might interact with an SH3 domain of a yet unknown protein). In addition, there is schematic representation of the predicted structure of Sans with 3 ankyrin repeats (these repeats associate to form higher order structures) and a SAM domain (found in signaling and nuclear proteins and appears to mediate cell–cell initiated signal transduction via the binding of SH2-containing proteins and in many cases mediates homodimerization, see the conserved domain data base at NCBI, http://www.ncbi.nlm.nih.gov/). Also shown is the predicted structure of cadherin 23 comprising 27 cadherin repeats which are involved in Ca^{2+}-dependent cell adhesion, one transmembrane domain and a cytoplasmic tail with no homology to any known intracellular functional domain. Cadherin 23 is thought to interact with the PDZ-2 domain of harmonin isoform b in the stereocilia (37, 73).

USH1D, *DFNB12* subjects and the waltzer mice have mutations in the gene encoding cadherin 23 (33, 34, 74). A summary of the known mutations of *CDH23*, identified in *USH1D* and *DFNB12* subjects, is given in Table 2. It can be seen that missense mutations, presumed hypomorphs, of *CDH23* cause non-syndromic recessive deafness while more severe mutant alleles (nonsense or truncating mutations) are associated with *USH1D* (34, 47). Some compound heterozygotes with one severe *CDH23* allele and a hypomorphic allele of *CDH23* have an Usher phenotype (47). No allelic variants of *CDH23* are reported which cause retinitis pigmentosa in individuals with normal hearing. No mutant alleles of mouse *Cdh23* are associated with age-related hearing loss or the modifier-of-deaf waddler, although the fine genetic mapping of *Cdh23*^{v} and *mdfw* supports the possibility that these loci are caused by allelic mutations (77).

CDH23 encodes a protein with 3354 amino acids that has 94% identity and 97% amino acid similarity with mouse cadherin 23. Cadherin 23 is predicted to have 27 ectodomains (or cadherin repeats which are involved in Ca^{2+}-dependent cell adhesion), a signal peptide at the amino terminus, one transmembrane domain and a unique cytoplasmic carboxy terminal tail (Fig. 1) (34, 74). Cadherins belong to a superfamily of glycosylated proteins typically mediating cell–cell adhesion via calcium-dependent interactions through homophilic ectodomains (78). In the inner ear, cadherin 23 is present along the entire developing stereocilia. Eventually its expression is restricted to the tip of the stereocilia (69). Cadherin 23 is found in the photoreceptor layer of developmental

stage P0 in mice (73) and presumably is essential for stereocilia formation (69, 73).

USH1F/DFNB23 and *Ames Waltzer* (*av*)

USH1F was mapped to chromosome 10q21-22, in a 15-cM interval centromeric to *DFNB12/USH1D* (27). *DFNB23*, an autosomal non-syndromic recessive deafness locus, maps to an interval overlapping *USH1F* (42). In the United States, *USH1D* and *USH1F* mutations combined are the second most common cause of Usher syndrome (50). Our data from Pakistani families supports this assessment (34, 35, 47, 67, see Table 1).

The mouse Ames waltzer (*av*) phenotype is due to a recessive mutation in Pcdh15 and is the mouse model for *USH1F*. Homozygote *Pcdh15* mutant mice are deaf with a degeneration of the inner ear neuroepithelium and vestibular dysfunction (79). *PCDH15* is the human ortholog and was proposed as a candidate for *USH1F* (79). Three different mutant alleles of *PCDH15* from four families segregating *USH1F* (35, 36) were recently reported. Protocadherin 15 has 11 ectodomains (cadherin repeats which are involved in Ca^{2+}-dependent cell adhesion), at least one transmembrane domain, a unique cytoplasmic carboxy domain (Fig. 2), and display 83% amino acid identity and 88% similarity with murine *Pcdh15* (35). It is widely expressed in inner and outer hair cells and in many other tissues from early development through adulthood (80). It is as yet unknown whether Pcdh15 is part of the same complex of proteins found interacting in the stereocilia of hair cells, but it does contain a class 1 PDZ interacting consensus sequence at the carboxy end.

In an ultrastructural analysis, the stereocilia degenerate and are disorganized, and for some alleles the stereocilia bundles were rotated 90° from their normal position (81). The endocochlear potential is normal in homozygous Ames waltzer (*av*^{J} and *av*^{2J}), excluding the involvement of the stria vascularis in the primary pathology (81). Protocadherin 15 is the second cadherin-like gene reported to cause deafness when mutated.

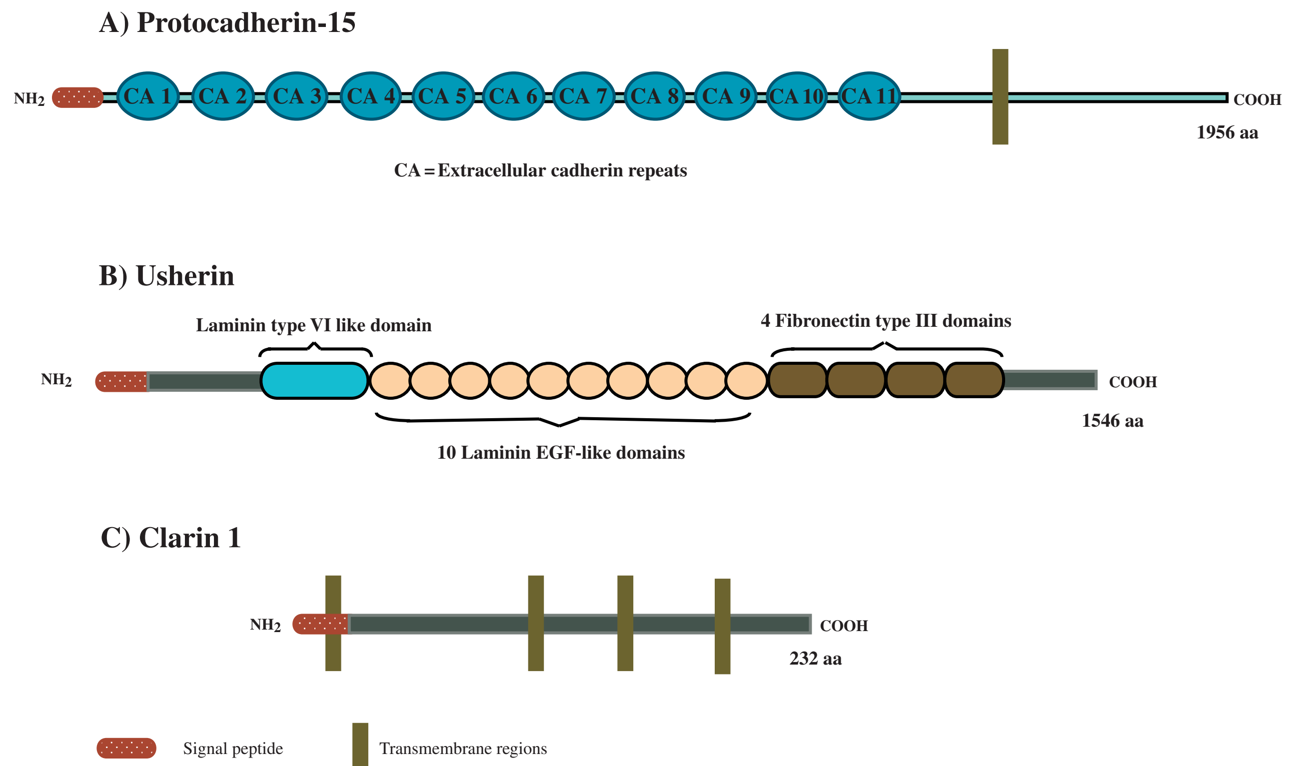

Fig. 2. (a) The domains of protocadherin 15 (PCDH15) predicted from 1955 amino acids. Protocadherin 15 has a signal peptide at the amino terminus, 11 extracellular calcium-binding domains, one predicted transmembrane domain and a unique carboxy terminus. TMpred and SMART predicted two transmembrane domains (1054–1072, and 1376–1397), TMHMM predicted only one transmembrane domain at 1376–1397 (35). (b) The predicted structure of usherin encoded by *USH2A*. There is a signal peptide followed by 285 amino acids of uncharacterized amino acid sequence, a laminin domain VI-like motif, 10 laminin type EGF-like modules, four fibronectin-like type III repeats and a unique carboxy terminus (38). (c) Schematic representation of clarin 1, recently identified by two different research groups, predicted to have three or possibly four transmembrane domains with different amino and carboxy termini from the previously identified isoform (40, 41).

USH1G and *Jackson shaker (js)*

USH1G maps to a 23-cM interval on chromosome 17q24-25 and was found segregating in a Palestinian family from Jordan (28). The mouse mutant Jackson shaker (*js*) (82) is linked to the syntenic region of mouse chromosome 11. Two different BACs, both containing *Sans* (scaffold protein containing ankyrin repeats and SAM domain, Fig. 1) found on the 72-kbp overlap in common between them, rescue the *js* phenotype. The two known *js* alleles harbor mutations in this gene (82) and four different mutant alleles of *SANS* were found segregating in *USH1G* linked families, confirming that *js* is the mouse homolog to *USH1G* (37). Sans protein is predicted to contain ankyrin repeats and a SAM domain. Both domains are known to mediate protein–protein interactions, hence the name scaffolding protein. Sans protein is also predicted to have a class-I PDZ-interacting consensus sequence and co-localizes with harmonin (a protein with several PDZ domains, see Fig. 1) upon co-transfection in HeLa cells (37), evidence that Sans could be part of the same molecular complex containing harmonin, myosin VIIa and caderin 23.

USH2A

USH2A was the first Usher syndrome locus to be identified (20). Subsequent analysis of additional USH2 families refined the linkage region to 1 Mb (83). Recent studies revealed that USH2 is the most common form of Usher syndrome and may account for more than 50% of all Usher subjects in some populations (9, 38). Moreover, *USH2A* could be responsible for as much as 85% of USH2 cases (84).

In 1998, a defect of the gene encoding a novel protein, Usherin, proved responsible for the *USH2A* phenotype (38). Usherin has 1546 amino acids (170–180 kDa) with 70% identity and 81% amino acid similarity with mouse usherin and the predicted protein structure has four main structural motifs (85). A predicted signal peptide (see Fig. 2) is followed by thrombospondin domain (Ts), laminin N-terminal domain (domain VI, LN), 10 laminin-type epidermal growth factor-like domains (LE) and four fibronectin type 3 domains (85). Usherin is a critical element of the basement membrane in the cochlea and retina, but is also expressed in many other tissues (86).

Mutational screening of *USH2A* subjects revealed 2299delG as a common and widespread mutation found in subjects from Europe, the United States, South Africa and China (85, 87, 88). Families with this mutation are described as either USH2 with moderate-to-severe hearing loss and normal vestibular function, or as atypical Usher families (similar to USH3) with progressive hearing loss and vestibular dysfunction (88). The frequency of this mutation in *USH2A* patients is high enough (approximately 20%) that it may be the single highest cause of retinitis pigmentosa in the human population (85), vying with Cys759Phe of the same gene causing as much as 4.5% of recessive retinitis pigmentosa without hearing loss (46). Since the initial identification of *USH2A*, more than 32 allelic variants have been reported (see Table 2).

USH3A

USH3A is common in eastern Finland and accounts for approximately 40% of Usher patients in that country (39). The chromosomal location of *USH3A* on chromosome 3q23-24 was determined from a genome-wide mapping effort on Finnish *USH3A* families (18). Linkage disequilibrium refined the location of the Finnish *USH3A* mutation to an interval of approximately 1 cM between markers D3S1299 and D3S3625 (89) and later to a genomic interval of only 250 kb (90). *USH3A* mutant alleles are not limited to Finland (84, 91–93).

Shotgun sequencing of two overlapping BACs in the *USH3A* linkage interval helped in the identification of a novel gene, *USH3A* (39). Mutations were found in *USH3A*, initially thought to have only four exons with an alternatively spliced first exon. Sequence analysis revealed the expected founder mutation (Y100X, or Fin_{major}) in the majority of the Finnish *USH3A* families (39). One other Finnish *USH3A* mutant allele is c.131T→A transversion at codon 44 in exon 2, resulting in M44K (Fin_{minor}, see Table 2) (39). Recently, two groups independently identified additional isoforms of *USH3A* using different strategies (40, 41). The longest identified transcript of *USH3A* has an exon upstream of the previously reported first exon along with exons 3 and 4, including the intervening intron between exons 3 and 4 (40, 41).

USH3A encodes the protein clarin-1 (see Fig. 2) with 232 amino acids and three or possibly four predicted transmembrane domains (40, 41). In mice, three different tissue-specific alternatively spliced isoforms of *Ush3A* were identified (40). The role of this gene in the cochlea and retina is yet to be determined, though it is postulated to participate in the synapse junctions between hair cells and cochlear ganglion cells because of a

weak similarity to stargazin (40). Additional human *USH3A* paralogs encoding clarin 2 and clarin 3 have been identified, located on chromosome 4p15.3 and 10q26.2, respectively. It is currently not known in what tissues these genes are expressed. They have more than 50% amino acid similarity with *USH3A* (clarin 1), and like *USH3A* contain three putative transmembrane domains (40).

Conclusion

Usher syndrome is by far the most common form of inherited deafness found in combination with retinitis pigmentosa. It is a heterogeneous disorder both clinically and genetically. Based on clinical phenotypes, Usher syndrome is classified into three main types, USH1, USH2 and USH3. USH1 is the most genetically heterogeneous with at least eight different loci. There are four loci for USH2 and at least two loci for USH3 (64). Of the 11 published loci, genes for seven have been identified as, *MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *SANS*, *USH2A* and *USH3A* encoding unconventional myosin VIIa, harmonin, cadherin 23, protocadherin 15, SANS, usherin and clarin 1, respectively. Five of these seven genes are expressed in the outer and inner hair cells of the organ of Corti while Usherin is known to be part of the extracellular basement membrane matrix and clarin-1 is expressed in the external sulcus, matrix of the limbus and spiral ligament (94).

Interactions between four USH1 proteins (myosin VIIa, harmonin b, SANS and cadherin 23) were recently demonstrated. The transmembrane complex formed by their interactions ensures the integrity of the stereocilia (69, 73). It would be interesting to determine the role, if any, that Pcdh15 has in this complex or in a similar complex as the hair cell phenotype of Pcdh15 mutants is very similar to cadherin 23, myosin VIIa and Sans mutant mice.

The most devastating aspect of Usher syndrome is progressive retinopathy. Interestingly, murine models of *USH1B*, *USH1D*, *USH1F* and now *USH1G* are reportedly deaf with vestibular hypofunction but they have no observed retinal degeneration (64). These mouse models are excellent for the study of hearing loss but not the pathophysiology of retinitis pigmentosa. The lack of a significant retinal phenotype in homozygous shaker 1, waltzer, Ames waltzer and Jackson shaker mice may indicate that these genes play different functions in the retina of mice as compared with humans, or perhaps there is compensation by a modifier gene in these strains that suppresses the retinal phenotype. Alternatively, in the mouse retina there may be functional redundancy by one of the other protein family members that can substitute for the absence of Myo7a, Cdh23, Pcdh15 or SANS. On the other hand, the absence of retinitis pigmentosa may provide models for the study of a redundant function in the mouse that permits retention of normal retinal function and, once defined, might be activated as therapy or prevention of retinitis pigmentosa in humans.

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