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.

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 Laguante 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...
| 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** |                      |                                 |      |         |             |                                                             |                                                             |
| A (95)     | 14q32               | –                              | DFNB2| MYO7A   | Shaker 1 (56) | Relative to USH1 49% (50)                                 | Relative to USH1 42.9%                                     |
| B (22)     | 11q13.5             | –                              | DFNB12| CDH23   | Waltzer (74)  | >2% (50)                                                    | 12.2%                                                       |
| C (23)     | 11p15.1             | –                              | DFNB18| USH1C   | Harmonin     | 33% (combined D&F, 50)                                     | 28.6%                                                       |
| D (26)     | 10q22.1             | –                              | DFNB14| CDH23   | Cadherin 23  | 33% (combined F&D, 50)                                     | 12.2%                                                       |
| E (96)     | 21q21               | –                              | DFNB23| SANS    | Protocadherin 15 | 16% (50)                                                    | 4.1%                                                        |
| F (27)     | 10q21.1             | –                              | SANS | SANS    | Jackson shaker (82) | Relative to USH2 >70% (85)                                 |                                                             |
| G (28)     | 17q25.1             | –                              | SANS | SANS    | –            |                                                             |                                                             |
|?           | Unknown             | –                              | –    | –       | –            |                                                             |                                                             |
| **USH type II** |                      |                                 |      |         |             |                                                             |                                                             |
| A (20)     | 1q41                | Non-syndromic RP(46)            | USH2A | Usherin | –            | Relative to USH2 >70% (85)                                 |                                                             |
| B (29)     | 3p23-24.2           | –                              | DFNB6| –       | –            |                                                             |                                                             |
| C (110)    | 5q14.3-21.1         | –                              | –    | –       | –            |                                                             |                                                             |
| ? (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 markers at 1q41 cosegregate with the phenotype in USH2 (Omaha, NE) families and markers at 11q13.5 cosegregate with the phenotype in Finnish families (24). It was then found that markers at 3q25 cosegregate with a locus at 11p15.1 (USH1B) was localized to chromosome 10q22.1 (26), USH1F was assigned the locus symbol USH1F (23, 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 (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 (shl) 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 shl (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).
| 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)       |
|        |       | 1198insT | MyTH4 domain            | (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)       |
|ogene| Gene| Position| Protein region|
|------|------|----------|---------------|
|IVS40-2| Pre-ERM domain| 99|
|P1887L| FERM domain| 99|
|2018delG| FERM domain| 99|
|2065delC| FERM domain| 62|
|2119-2215del2 kb| FERM domain| 62|
|G2137E| FERM domain| 96|
|G2163S| FERM domain| 96|
|G2167D| FERM domain| 101|
| | Atypical USH1B| L651P cpd. het. w/R1602Q|
| | Motor domain and FERM domain| 49|
| | USH1C| IVS1 + 1 G > T|
| | Amino terminus| 104|
| | R31X| Amino terminus| 104|
| | 216G > A| Amino terminus| 31|
| | 238-239insC| Amino terminus| 31, 32, 104|
| | IVS5 + 1 G > A| PDZ1| 104|
| | IVS5-2delA| PDZ1| 32|
| | IVS8 + 2T > G| PDZ2| 67|
| | 769-770ins36| PDZ2| 67|
| | G431V| Coiled coil| 68|
| | 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|
| | D1848N| 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|
| | IVS5 + 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|
| | T103ST| CA10| 47|
| | delM1281| CA12| 33|
Table 2. Continued

| Gene       | Mutation | Protein Region | Ref. |
|------------|----------|----------------|------|
| PCDH15     |          |                |      |
| USH1F      | R3X      | Signal peptide | (35, 36) |
|            | R245X    | CA2            | (111) |
|            | 1471delT | CA3            | (36)  |
|            | IVS27-2A| CA11           | (35)  |
| SANS       | 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      | 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) |
|            | 4338-9delICT | 4th Fibronectin type III | (38) |
|            | 4510-4511insA | Carboxy tail region | (85) |
|            | T1515M   | Carboxy tail region | (108) |
|            | C759F    | 5th laminin EGF-like | (46)  |
| RP without HL |          |                |      |
| USH3A      | N48K     | Pre-1st TM      | (40, 41) |
|            | 149delCAGG/insTGTG-CAA | 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.
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 Cdhl23 are associated with age-related hearing loss or the modifier-of-deaf waddler, although the fine genetic mapping of Cdhl23 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), 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).

*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).
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<sup>2+</sup>-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<sup>1</sup> and av<sup>2</sup>), 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.

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**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).
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**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 Finmajor) 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 (Finminor, 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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