

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Usher Syndrome: Genes, Proteins, Models, Molecular Mechanisms, and Therapies

Jun Yang

*Department of Ophthalmology and Visual Sciences, Moran Eye Center, University of Utah
USA*

1. Introduction

Usher syndrome (USH) is an autosomal recessive genetic disease, characterized by both deafness and blindness. It was first described by Albrecht von Graefe, a German ophthalmologist, in 1858 (von Graefe, 1858) and then named after Charles Usher, a British ophthalmologist, who reported the inheritance of this disease on the basis of 69 cases in 1914 (Usher, 1914). USH is clinically heterogeneous and is categorized into three types, according to the severity of its hearing and vestibular symptoms (Smith et al., 1994; Petit, 2001). Type I (USH1) patients have congenital severe to profound deafness as well as vestibular dysfunction; Patients with USH2 exhibit congenital moderate degree of hearing loss and normal vestibular function; and those with USH3 display progressive hearing impairment and occasional vestibular dysfunction. The vision problem of all three types is manifested as retinitis pigmentosa (Hartong et al., 2006; Sadeghi et al., 2006; Fishman et al., 2007; Sandberg et al., 2008; Malm et al., 2011), showing early night and peripheral vision loss and eventual central vision loss.

USH is the most common genetic cause of combined blindness and deafness, occurring in about 1 in 23,000 people worldwide (Boughman et al., 1983; Keats and Corey, 1999; Hartong et al., 2006). It represents 50% of the blindness-deafness cases, 5% of all congenital deafness and 18% of retinitis pigmentosa (Millan et al., 2011). In Europe, USH1, USH2 and USH3 generally account for 25-44%, 56-75%, and 2% of all USH cases, respectively (Grondahl, 1987; Hope et al., 1997; Rosenberg et al., 1997; Spandau and Rohrschneider, 2002). Due to the regional founder effect, USH3 is much more common in Birmingham and Finland (Pakarinen et al., 1995; Hope et al., 1997). To date, there is no cure for this disease. USH patients mainly rely on early diagnosis and early education to adapt themselves to their dual sensory loss.

2. USH genes

USH is genetically diverse besides its clinical heterogeneity. Currently, eleven loci have been identified (Hereditary hearing loss homepage and Hmani-Aifa et al., 2009), and nine genes on these loci are known. Among these genes, five are involved in USH1, three in USH2 and one in USH3 (Reiners et al., 2006; Williams, 2008; Millan et al., 2011). Although the functions of some USH genes are relatively clear now in the inner ear (see section 6), extensive work is still necessary to elucidate the functions of USH genes in both the inner ear and the retina.

2.1 USH1 genes

In the past 20 years, seven loci have been assigned to USH1. They are *USH1B-H*. *USH1A* was first localized on 14a32.1 from a study in nine USH1 families in the Poitou-Charentes region of France, and was recently withdrawn due to the discovery that most of these families in fact carry mutations on the *USH1B* locus (Gerber et al., 2006). The genes underlying *USH1B*, *USH1C*, *USH1D*, *USH1F*, and *USH1G* have been identified as *MYO7A* (myosin VIIa) (Weil et al., 1995), *USH1C* (harmonin) (Bitner-Glindzicz et al., 2000; Verpy et al., 2000), *CDH23* (cadherin 23) (Bolz et al., 2001; Bork et al., 2001), *PCDH15* (protocadherin 15) (Ahmed et al., 2001; Alagramam et al., 2001b), and *USH1G* (SANS) (Weil et al., 2003), respectively. Among them, *MYO7A*, *USH1C*, *CDH23* and *PCDH15* are also the causative genes for nonsyndromic deafness, *DFNB2/DFNA11* (Liu et al., 1997; Weil et al., 1997), *DFNB18* (Ahmed et al., 2002), *DFNB12* (Bork et al., 2001), and *DFNB23* (Ahmed et al., 2003), respectively. The *USH1E* and *USH1H* loci were mapped to chromosome 21q21 and 15q22-23 (Chaib et al., 1997; Ahmed et al., 2009). However, the genes at these loci have not yet been pinpointed.

MYO7A is the most prevalent gene causing USH1 (Astuto et al., 2000). It encodes an unconventional actin-based motor protein with the conserved motor domain and five IQ motifs (Figure 1A). These domains are responsible for binding to actin, ATP, and myosin light chain. Therefore, *MYO7A* may move its cargos along the actin filaments using the energy generated from the hydrolysis of ATP. However, the motor domain of *MYO7A* shows a strong affinity to ADP and, thus, stays bound to actin filament for a long time (Heissler and Manstein, 2011). In this case, *MYO7A* may be involved in generating tensions between two proteins or cellular structures. The tail of *MYO7A* has a series of protein-protein interaction domains, including a single α -helix (SAH), a coiled-coil domain (CC), two myosin tail homology 4 domains (MyTH4), two band 4.1, ezrin, radixin, moesin domains (FERM), and a src homology 3 domain (SH3) (Figure 1A). These domains are thought to be engaged in binding to cargos and/or anchoring to proteins.

Harmonin (also known as AIE-75 or PDZ-73) is expressed in many different tissues (Kobayashi et al., 1999; Scanlan et al., 1999). Nine transcripts have so far been discovered (Verpy et al., 2000; Reiners et al., 2003). They are categorized into three groups, isoforms a, b and c (Figure 1B). All these isoforms have multiple PDZ (postsynaptic density 95; discs large; zonula occludens-1) domains and at least one CC domain. The CC domain is reported to participate in harmonin dimerization (Adato et al., 2005b), and the PDZ domain is well known to interact with PDZ-binding motifs (PBMs) in other proteins (Sheng and Sala, 2001). Isoform b specifically has a proline, serine and threonine-rich (PST) domain. This domain has been demonstrated to bind and bundle actin filaments (Boeda et al., 2002). In summary, harmonin may organize a multi-protein complex and attach this complex to actin filaments.

CDH23 and *PCDH15* both have multiple transcripts and are grouped into isoforms a, b and c for *CDH23* (Lagziel et al., 2005; Lagziel et al., 2009) and isoforms CD1, CD2, CD3 and SI for *PCDH 15* (Ahmed et al., 2006) (Figures 1C and 1D). As the distant members of the classical cadherin superfamily, the proteins of these two genes have various repeats of extracellular cadherin (EC) domains in their extracellular regions. Accordingly, it has been proposed and supported by many studies in hair cells (see below) that the two proteins function in cell adhesion through their homophilic and heterophilic interactions. The two proteins probably anchor to the intracellular structures through the PBMs in their cytoplasmic regions (Figures 1C and 1D).

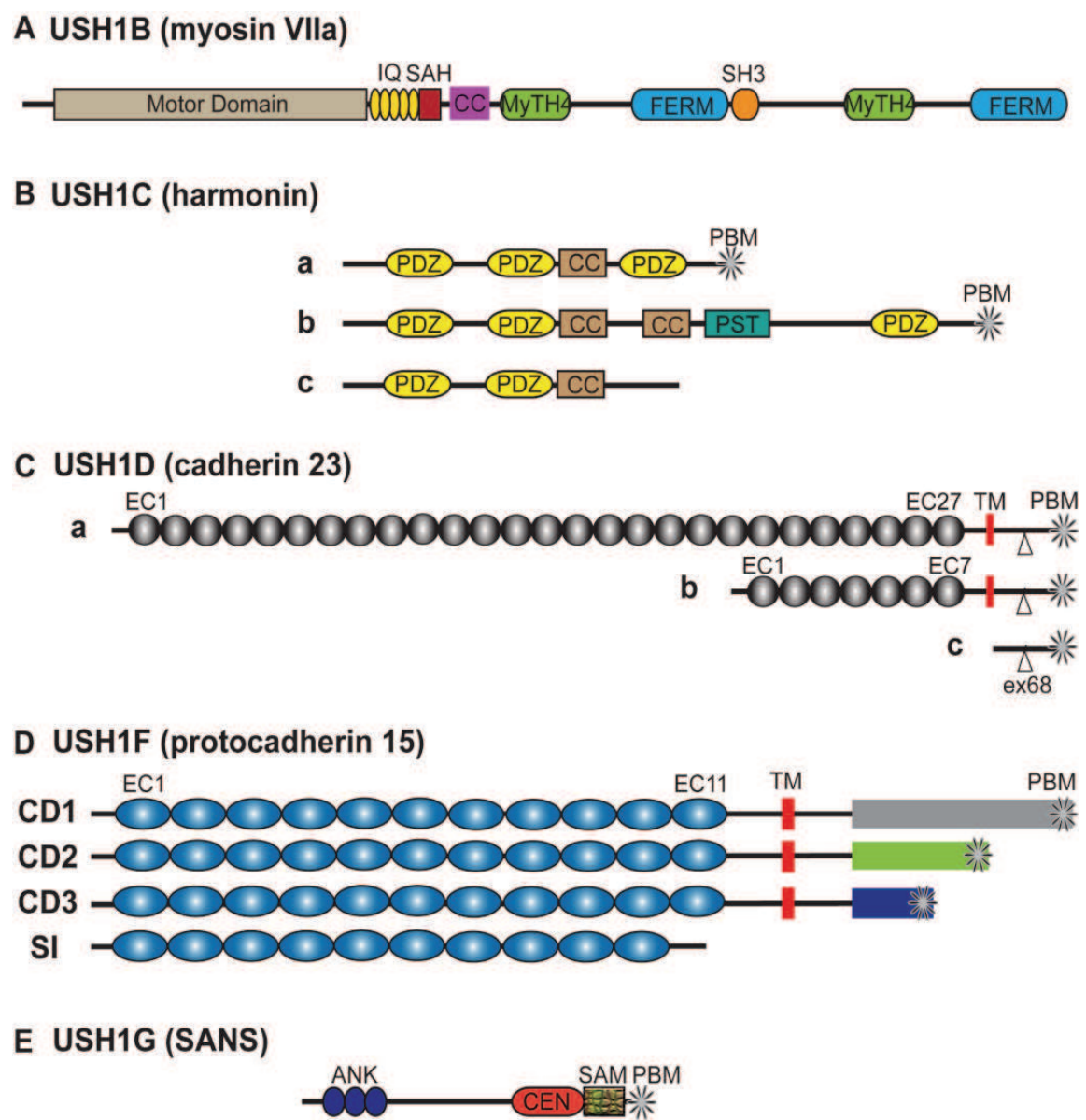


Fig. 1. Domain structures of USH1 proteins

Mutations in SANS are rare in USH1 patients. Some mutations, such as c.1373 A>T and c.163_164 + 13del15, cause the clinical symptoms close to USH2 (Kalay et al., 2005; Bashir et al., 2011). The protein of this gene consists of several putative protein-protein interaction domains, including three ankyrin -like (ANK) repeats, a central (CEN) domain, a sterile alpha motif (SAM) and a PBM (Figure 1E). Therefore, like harmonin, SANS is believed to be a putative scaffold protein.

2.2 USH2 genes

Four USH2 loci were originally defined, *USH2A-D*. The genes responsible for *USH2A*, *USH2C*, and *USH2D* are *USH2A* (usherin) (Eudy et al., 1998), *GPR98* (G Protein-coupled Receptor 98) (Weston et al., 2004), and *WHRN* (whirlin) (Ebermann et al., 2006), respectively. The gene for *USH2B* was once considered to be *NBC3* (sodium bicarbonate cotransporter)

(Bok et al., 2003). However, further study of the consanguineous Tunisian family carrying the *USH2B* locus demonstrates that mixed mutations in the *GPR98* and *PDE6B* genes are responsible for the disease manifestation in the family and, thus, the *USH2B* locus was withdrawn (Hmani-Aifa et al., 2009). Moreover, a novel *USH2* locus has recently been localized on the chromosome 15q, though the underlying gene has not been identified so far (Ben Rebeh et al., 2008). ‘

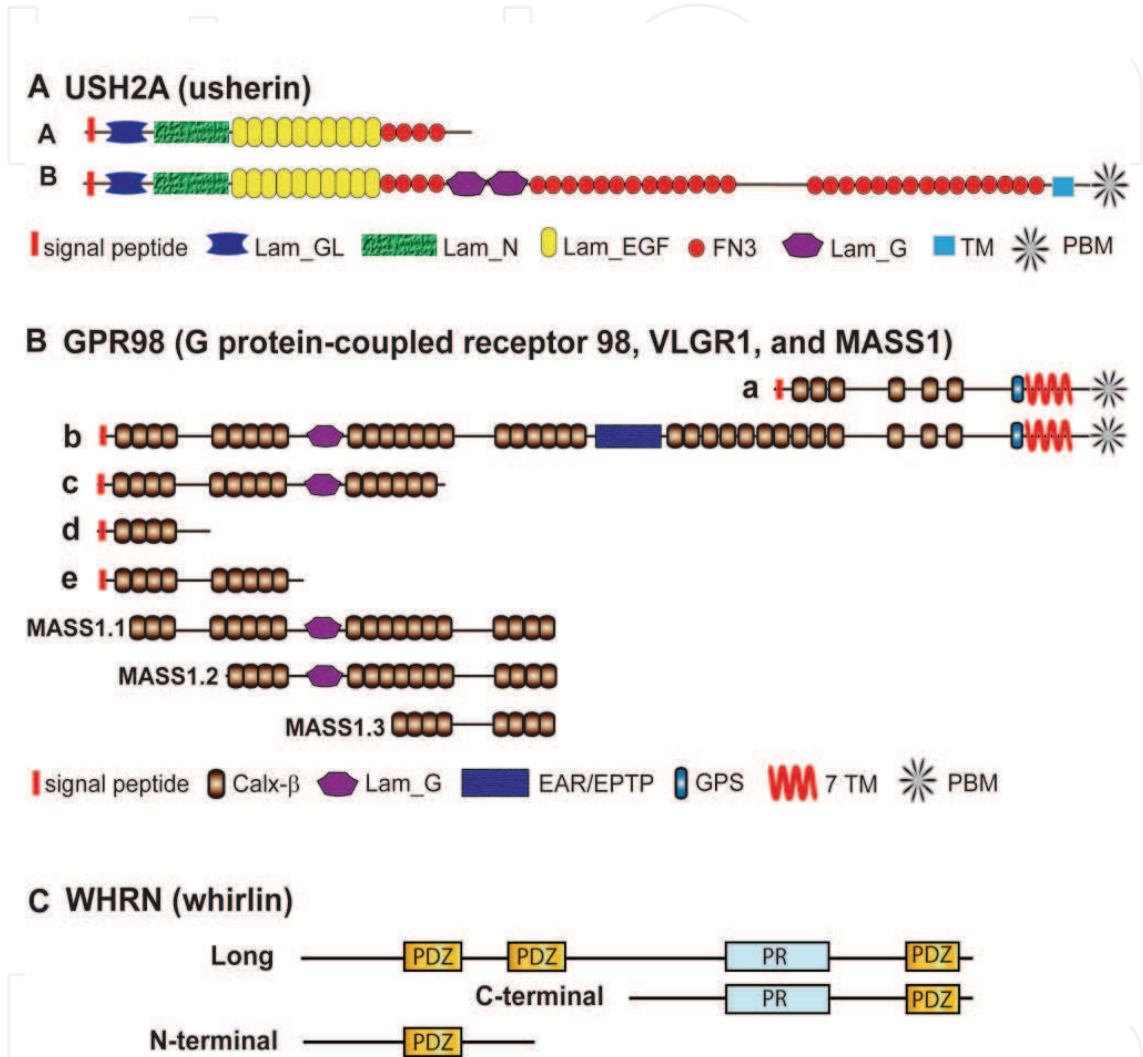


Fig. 2. Domain structures of USH2 proteins

USH2A is the most predominant causative gene in all USHs among different human ethnic populations (Eudy et al., 1998; Dreyer et al., 2000; Weston et al., 2000; Aller et al., 2004; van Wijk et al., 2004; Adato et al., 2005a; Hartong et al., 2006; Baux et al., 2007; Kaiserman et al., 2007; Dreyer et al., 2008; Nakanishi et al., 2009; Yan et al., 2009; McGee et al., 2010). Its mutations lead to a wide spectrum of vision and hearing defects in patients. Some *USH2A* mutations, such as p.C759F and p.G4674R, are known to cause only nonsyndromic retinitis pigmentosa (Rivolta et al., 2002; Seyedahmadi et al., 2004; Kaiserman et al., 2007). *USH2A* has 72 exons and is expressed as isoforms A and B (Figure 2A). Isoform B, the major isoform in the retina (Liu et al., 2007), is an extremely large transmembrane protein with 5202 amino acids (aa) in humans (van Wijk et al., 2004). Its long extracellular region has repeated various

laminin (Lam) and fibronectin III (FN3) functional domains common in cell adhesion proteins and extracellular matrix proteins. Its cytoplasmic region has a PBM. Isoform A is an N-terminal 1546-aa fragment of isoform B. USH2A is thought to be involved in cell adhesion.

The *GPR98* gene, also known as *VLGR1* (Very Large G protein-coupled Receptor 1) and *MASS1* (Monogenic Audiogenic Seizure Susceptibility 1), exists only in the vertebrate (Gibert et al., 2005) and is one of the largest genes, with 90 exons (McMillan et al., 2002). Its mRNA is present mostly in the brain and spinal cord during development (McMillan et al., 2002; Weston et al., 2004), but it can also be found in many other tissues (Nikkila et al., 2000; Skradski et al., 2001; McMillan et al., 2002; Weston et al., 2004). *GPR98* expresses multiple mRNA transcripts, including isoforms a, b and c in humans and isoforms b, d, e and Mass1 in rodents (Figure 2B) (Nikkila et al., 2000; Skradski et al., 2001; McMillan et al., 2002; Yagi et al., 2005). Mutations in the longest isoform, isoform b, have been identified in patients with USH2C (Weston et al., 2004; Ebermann et al., 2009; Hilgert et al., 2009). Additionally, different mutations along the murine *Gpr98* gene share common phenotypes in vision and hearing (Skradski et al., 2001; McMillan and White, 2004; Johnson et al., 2005; Yagi et al., 2005; McGee et al., 2006; Michalski et al., 2007; Yagi et al., 2007). These findings suggest that isoform b is the major isoform in both the retina and the inner ear and is essential for vision and hearing. This isoform is 6306 aa long in humans. It has signature domains of family B of G protein-coupled receptors (GPCRs), i.e., a GPCR proteolytic site (GPS) and a 7-transmembrane domain (7TM). Therefore, GPR98 may function in signal transduction. GPR98 also has a PBM at its C-terminus. Along its long extracellular region, it has a laminin globular-like domain (LamG_L), an epilepsy associated repeat (EAR)/epitempin (EPTP) domain, and multiple tandem-arranged Calx β domains. While the function of EAR/EPTP is unknown, LamG_L is a cell adhesion domain, and the Calx β domain is able to bind to Ca²⁺ with low affinity in vitro (Nikkila et al., 2000; McMillan and White, 2011).

Mutations of whirlin cause either USH2D or nonsyndromic deafness, *DFNB31*. Interestingly, mutations at the N-terminal half of the gene, such as p.P246HfxX13 and compound heterozygosity of p.Q103X and c.837+1G>A, are manifested as USH2D (Ebermann et al., 2006; Audo et al., 2011), while mutations at the C-terminal half, such as p.R778X and c.2423delG, were found in patients with *DFNB31* (Mburu et al., 2003; Tlili et al., 2005). Whirlin has multiple mRNA transcripts in the inner ear and the retina (Mburu et al., 2003; Belyantseva et al., 2005; van Wijk et al., 2006; Yang et al., 2010), which can be conceptually translated into three groups of proteins, the long, N-terminal, and C-terminal isoforms (Figure 2C). The long isoform contains three PDZ domains and a proline-rich region (PR). Thus, whirlin is a homolog of harmonin. At the protein level, whirlin mainly expresses the long isoform in the retina and the long and C-terminal isoforms in the inner ear (Yang et al., 2010). Because both the PDZ domain and PR region are protein interaction modules, whirlin is believed to be implicated in the assembly of multi-protein complexes at specific subcellular locations, similar to harmonin.

2.3 USH3 and USH related genes

The only gene currently identified in USH3 is clarin-1 for the *USH3A* locus (Joensuu et al., 2001; Adato et al., 2002; Fields et al., 2002). Like other USH genes, clarin-1 has multiple transcript variants due to different splicings and usages of transcription start sites (Vastinsalo et al., 2010). The primary transcript encodes a protein with four predicted

transmembrane domains and a C-terminal potential PBM (Figure 3). Clarin-1 shows a sequence homologous to stargazin, an auxiliary subunit of ion channels in the synapse (Osten and Stern-Bach, 2006; Tomita et al., 2007). Presently, several research groups are intensively focusing on understanding this gene (Aarnisalo et al., 2007; Geller et al., 2009; Geng et al., 2009; Tian et al., 2009; Zallocchi et al., 2009). However, the biological function and cellular expression of clarin-1 still remain elusive.



Fig. 3. Domain structure of USH3A

Recently, *PDZD7* was shown to be a modifier gene for the retinal symptom in *USH2A* patients and, together with *USH2A* or *GPR98*, to contribute to a digenic USH form (Ebermann et al., 2010). Interestingly, this newly identified USH modifier and contributor gene is also a homolog of harmonin. It has several isoforms (Schneider et al., 2009; Ebermann et al., 2010). The long isoform has three PDZ domains and one PR region. The two short isoforms are the N-terminal fragments of the long isoform with only two PDZ domains. However, the short isoforms have not been confirmed at the protein level. Similar to both harmonin and whirlin, different mutations in *PDZD7* are involved in either USH or nonsyndromic deafness. A homozygous reciprocal translocation, 46,XY,t(10;11)(q24;q23), was found to disrupt the *PDZD7* gene at intron 10, which causes nonsyndromic congenital hearing impairment (Schneider et al., 2009). A heterozygous p.R56PfsX mutation of *PDZD7* was found to exacerbate retinal degeneration in an *USH2A* patient, compared to her sibling carrying the same *USH2A* mutation. Additionally, the heterozygous mutations of *PDZD7*, c.1750-2A>G and p.C732LfsX, are present in USH patients with a heterozygous *USH2A* mutation, p.R1505SfsX, and with a heterozygous *GPR98* mutation, p.C732LfsX, respectively (Ebermann et al., 2010).

3. Animal models

Numerous spontaneous and transgenic USH animal models are now available. Table 1 lists the detailed information about the mouse models. The majority of these models show congenital hearing loss as expected. However, only a few of them, *Ush1c* knockin, *Ush2a* knockout, and whirlin knockout mice, manifest obvious widespread retinal degeneration. *Ush1c^{dfcr}* mice on some specific genomic background and *Myo7a^{4626SB}* and *Cdh23^V* double mutant mice show only slight retinal degeneration (Johnson et al., 2003; Lillo et al., 2003; Williams et al., 2009). Among the rest of the USH mouse models, some *Myo7a*, *Cdh23*, *Pcdh15*, and *Grp98* mutant strains show abnormal electroretinogram (ERG) responses but no retinal degeneration (Libby and Steel, 2001; Libby et al., 2003; Haywood-Watson et al., 2006; McGee et al., 2006), indicating that the function of photoreceptors is compromised. The reasons for the discrepancy between USH patient symptoms and USH mutant mouse phenotypes are largely unclear. Many factors could contribute to this, such as the gene isoform composition, mutation type and position in the genes, genomic background, redundant protein compensation, photoreceptor structure and physiology, influence of non-genetic factors, sensitivity of diagnostic measures, etc. (El-Amraoui and Petit, 2005). Additionally, although retinitis pigmentosa in USH is characterized to have an onset before

or during puberty (Smith et al., 1994; Petit, 2001), more and more atypical USH patients have been found (Edwards et al., 1998; Sadeghi et al., 2006; Cohen et al., 2007; Fishman et al., 2007; Sandberg et al., 2008; Malm et al., 2010.; Bashir et al., 2011). These patients have relatively late onset vision loss, which may explain the lack of retinal phenotype in most USH mutant mice, whose lifespan is only about two years.

Zebrafish models for several USH genes have also been reported, including mariner (*myo7a*), *ush1c*, sputnik (*cdh23*), and orbiter (*pcdh15*) (Phillips et al., 2011; Nicolson et al., 1998; Ernest et al., 2000; Sollner et al., 2004; Seiler et al., 2005). Defects in hearing, balance, and vision are manifested during the early life in two *ush1c* mutants. Interestingly, zebrafish has two orthologs of *PCDH15*. Disruption of one leads to the auditory and vestibular dysfunction, while disturbance of the other results in defects in the photoreceptor structure and retinal function. Mariner exhibits similar phenotypes to *Myo7a* mice in hearing, balance and vision. Sputnik has problems with the auditory and vestibular system, but its vision phenotype has not been reported. Currently, studies on other USH genes in zebrafish using the morpholino knockdown technique are being actively pursued (Ebermann et al., 2010). Moreover, a rat model with a point mutation leading to premature truncation of *Myo7a* was generated by N-ethyl-N-nitrosourea mutagenesis and named Tornado (Smits et al., 2005). In this model, hearing but not vision defects have been characterized. Therefore, exploration of USH genes in more vertebrate organisms will provide additional ways to understand the biological functions of these genes, in particular, in the retina.

Model name	Mutations	Phenotypes	References
USH1			
<i>Myo7a</i>			
<i>Myo7a^{sh1}</i>	p.R502P	Circling, head tossing, hearing impairment	(Mburu et al., 1997; Libby and Steel, 2001)
<i>Myo7a^{6J}</i>	p.R241P	Circling, head tossing, deafness	(Mburu et al., 1997; Libby and Steel, 2001)
<i>Myo7a^{26SB}</i>	p.F1762I	Circling, head tossing, deafness	(Mburu et al., 1997; Libby and Steel, 2001)
<i>Myo7a^{816SB}</i>	p.L646_Q655del	Circling, head tossing, deafness, reduced ERG	(Mburu et al., 1997; Libby and Steel, 2001)
<i>Myo7a^{3336SB}</i>	p.C2144X	Circling, head tossing, deafness	(Mburu et al., 1997; Libby and Steel, 2001)
<i>Myo7a^{4494SB}</i>	p.A246fs?X5	Circling, head tossing, deafness	(Mburu et al., 1997; Liu et al., 1999; Libby and Steel, 2001)
<i>Myo7a^{4626SB}</i>	p.Q720X	Circling, head tossing, deafness, reduced ERG	(Mburu et al., 1997; Libby and Steel, 2001)

Model name	Mutations	Phenotypes	References
<i>Myo7a^{7l}</i>	p.A1363AfsX27	Circling, head tossing, deafness, reduced ERG	(Mburu et al., 1997; Libby and Steel, 2001; Yang et al., 2011)
<i>Myo7a^{Hdb}</i>	p.I178F	Circling, head tossing, low-frequency hearing impairment	(Rhodes et al., 2004)
<i>Myo7a^{8l}</i>	Not known	Circling, head tossing, deafness, reduced ERG	(Mburu et al., 1997; Libby and Steel, 2001)
<i>Myo7a^{9l}</i>	Not known	Circling?, head tossing?, deafness?, reduced ERG	(Mburu et al., 1997; Libby and Steel, 2001)
Harmonin			
<i>Ush1c</i> knockout	Replacement of exons 1-4 with β -gal/neo cassette	Circling, head tossing, deafness	(Tian et al., 2010)
<i>Ush1c^{dfcr}</i>	A deletion involving exons 12-15, A-D	Circling, head tossing, deafness, slight retinal degeneration at 9 months of age	(Johnson et al., 2003)
<i>Ush1c^{dfcr-2l}</i>	One bp deletion in exon C	Circling, head tossing, deafness	(Johnson et al., 2003)
<i>Ush1c^{tm1.1Ugds}</i>	Exon 1 deletion	Circling, head tossing, deafness	(Lefevre et al., 2008)
<i>Ush1c</i> knockin	c.216G>A	Circling, head tossing, deafness, retinal degeneration	(Lentz et al., 2007; Lentz et al., 2010)
<i>Ush1c</i> -PDZ2 ^{AAA}	Replacement of GLG (221-223aa) in PDZ2 with AAA	Hair bundle defect	(Grillet et al., 2009)
Cdh23			
<i>jera</i>	p.V2360E	deafness	(Manji et al., 2011)
<i>erlong</i>	p.S70P	Circling, head tossing, deafness	(Han et al., 2010)
<i>salsa</i>	p.E737V	Circling, head tossing, deafness	(Schwander et al., 2009)
<i>Cdh23^v</i>	p.N279EfsX39	Circling, head tossing, deafness, reduced ERG responses	(Wilson et al., 2001; Libby et al., 2003)
<i>Cdh23^{v-l}</i>	p.E1169NfsX7	Circling, head tossing, deafness	(Wilson et al., 2001)
<i>Cdh23^{v-2l}</i>	c.4104 + 1G>A	Circling, head tossing, deafness, faster ERG responses	(Di Palma et al., 2001b; Libby et al., 2003)

Model name	Mutations	Phenotypes	References
<i>Cdh23</i> ^{V-3J}	p.W1764X	Circling, head tossing, deafness	(Di Palma et al., 2001a)
<i>Cdh23</i> ^{V-4J}	p.N2718del3	Circling, head tossing, deafness	(Di Palma et al., 2001a)
<i>Cdh23</i> ^{V-5J}	p.R2935X	Circling, head tossing, deafness	(Di Palma et al., 2001a)
<i>Cdh23</i> ^{V-6J}	p.E302X	Circling, head tossing, deafness	(Di Palma et al., 2001b)
<i>Cdh23</i> ^{V-7J}	p.Y1197MfsX47	Circling, head tossing, deafness	(Di Palma et al., 2001a)
<i>Cdh23</i> ^{V-ngt}	p.G49VfsX3	Circling, head tossing, deafness	(Wada et al., 2001)
<i>Cdh23</i> ^{V-Alb}	c.1635C>Tdel119	Circling, head tossing, deafness, normal ERG responses	(Di Palma et al., 2001b; Libby et al., 2003)
<i>Cdh23</i> ^{Vbus}	c.9633 + 1G>A	Circling, head tossing, deafness	(Yonezawa et al., 2006)
<i>Cdh23</i> ^{Ahl}	c.753G>A	Susceptibility to age-related hearing loss	(Noben-Trauth et al., 2003)
<i>Pcdh15</i>			
<i>Pcdh15</i> ^{av-1}	p.A645_K922del	Circling, head tossing, deafness, normal retinal function	(Alagramam et al., 2001a; Ball et al., 2003)
<i>Pcdh15</i> ^{av-2J}	p.D31_N57del	Circling, head tossing, deafness, normal retinal function	(Alagramam et al., 2001a; Ball et al., 2003)
<i>Pcdh15</i> ^{av-3J}	p.E1373RfsX36	Circling, head tossing, deafness, normal retinal function	(Alagramam et al., 2001a; Ball et al., 2003)
<i>Pcdh15</i> ^{av-5J}	IVS14-2A>G	Circling, head tossing, deafness, reduced ERG responses	(Washington et al., 2005; Haywood-Watson et al., 2006)
<i>Pcdh15</i> ^{av-6J}	p.G962_K1008del	Circling, head tossing, deafness	(Alagramam et al., 2011)
<i>Pcdh15</i> ^{av-Jfb}	p.D701GfsX17	Circling, head tossing, deafness, reduced ERG responses	(Hampton et al., 2003; Haywood-Watson et al., 2006)
<i>Pcdh15</i> ^{av-TgN2742Rptw}	A large insertion	Circling, head tossing, deafness, normal retinal function	(Alagramam et al., 2001a; Ball et al., 2003)
<i>Sans</i>			
<i>Ush1g</i> ^{js}	p.E228RfsX8	Circling, head tossing, deafness	(Kikkawa et al., 2003)

Model name	Mutations	Phenotypes	References
<i>Ush1g^{js-2l}</i>	p.L81GfsX103	Circling, head tossing, deafness	*
<i>Ush1g^{F1}</i>	Exon 2 flanked with FRT sites	Hearing defects after deletion of exon 2	(Caberlotto et al., 2011)
USH2			
<i>Ush2a</i>			
<i>Ush2a</i> knockout	replacement of exon 5 with a neomycin ^r cassette	hearing impairment, retinal degeneration	(Liu et al., 2007)
<i>Gpr98</i>			
<i>Gpr98</i> knockout	replacement of exons 2-4 with a neomycin ^r cassette	audiogenic seizure susceptibility, hearing impairment	(Yagi et al., 2005; Michalski et al., 2007)
<i>Gpr98</i> -EYFP knockin	replacement of exons 2-4 with a EYFP-neomycin ^r cassette	defects in hair cell stereocilia	(Yagi et al., 2007)
Frings & BUB/BnJ	a G deletion at 6864 bp (NM_054053) causing a p.V2250X mutation	audiogenic seizure susceptibility, hearing impairment	(Skradski et al., 2001; Johnson et al., 2005)
<i>Gpr98</i> /del7TM	replacement of exon 82 with a HA-neomycin ^r cassette	audiogenic seizure susceptibility, hearing impairment, mildly abnormal ERG responses	(McMillan and White, 2004; McGee et al., 2006)
<i>Whrn</i>			
<i>Whrn</i> knockout	partial replacement of exon 1 with a neomycin ^r cassette	hearing impairment, retinal degeneration	(Yang et al., 2010)
whirler	a 592-bp deletion causing a p.H433fsX58 mutation	hearing impairment, no retinal degeneration	(Lane, 1963; Holme et al., 2002; Mburu et al., 2003; Yang et al., 2010)
USH3			
<i>Ush3a</i>			
<i>Ush3a</i> knockout	Disruption and deletion of promoter and exon 1	Circling, head tossing, deafness	(Geller et al., 2009)

MYO7A: NP_032689, CDH23: NP_075859, PCDH15: NP_075604, SANS: NP_789817

*: our unpublished data.

Table 1. USH mutant mouse models

4. Cellular localization of USH proteins

Defects in USH proteins result in Usher syndrome, nonsyndromic deafness, or retinitis pigmentosa, indicating that these proteins are essential in the inner ear and the retina. Therefore, extensive efforts have been put to investigate the cellular location of these proteins in these two tissues. The cellular localization of USH proteins in other tissues is relatively unclear, although some USH proteins are known to be present in the kidney, colon, brain, lung, olfactory neuron, ovary, oviduct, testes and intestine (el-Amraoui et al., 1996; Hasson et al., 1997; Wolfrum et al., 1998; Kobayashi et al., 1999; Scanlan et al., 1999; Bhattacharya et al., 2002; Pearsall et al., 2002).

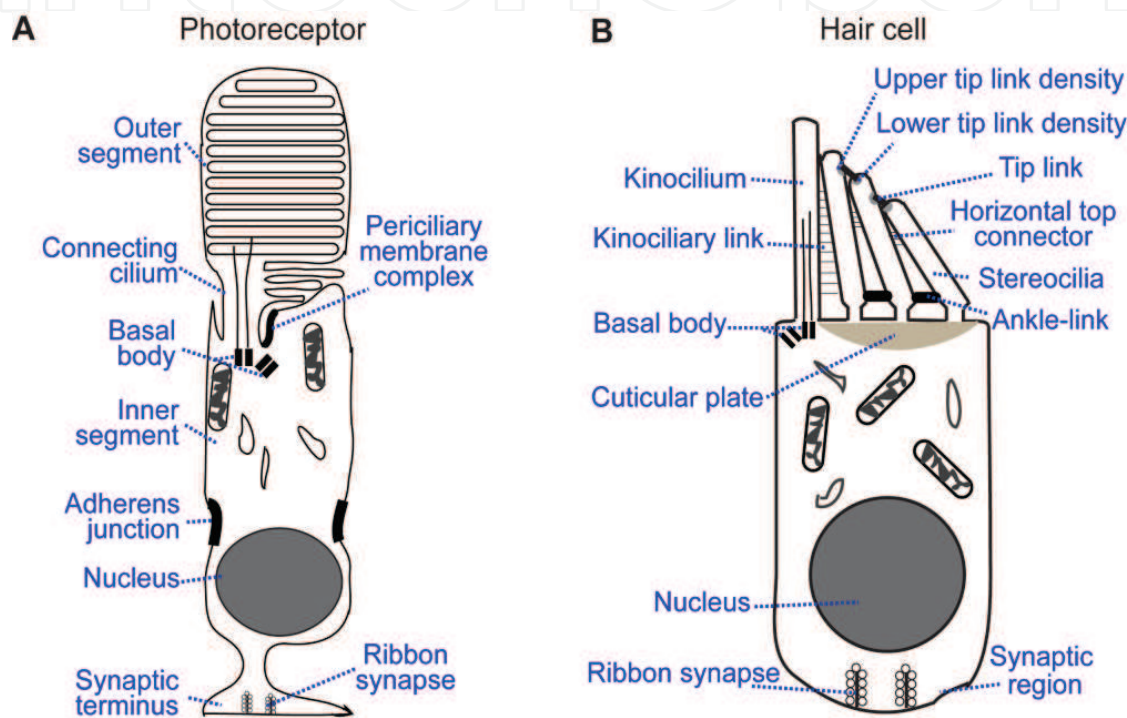


Fig. 4. Schematic diagrams of a rod photoreceptor and a hair cell

4.1 USH proteins in the inner ear

The inner ear is composed of the cochlea and vestibular system for hearing and balance, respectively. In the vestibular system, hair cells exist in the maculae of the saccule and utricle and the cristae ampullares of the semicircular canals. In the cochlea, one row of inner hair cells and three rows of outer hair cells exist in the organ of Corti. The inner hair cells are responsible for mechanoelectric transduction, whereas the electromotile outer hair cells also perform an electromechanical transduction, thereby amplifying the sound-evoked vibrations of the entire sensory epithelium (Leibovici et al., 2008). All types of hair cells have stereocilia on their apical surfaces, which are modified microvilli filled with bundles of actin filaments. The stereocilia are well-organized into rows of different lengths and form a staircase-like hair bundle (Figure 4). Along with the hair bundle, there exists a real cilium, called kinocilium, which is filled with microtubules. Various links have been discovered along the entire length of the stereocilia and the kinocilium during development and in

adulthood (Goodyear and Richardson, 1999; Goodyear and Richardson, 2003; Goodyear et al., 2005).

The distribution of USH proteins in hair cells vary dramatically from the emergence of stereocilia to their maturation. All USH1 proteins are present either at the tip, the ankle links, the transient lateral links, or the kinociliary links of the stereocilia during the early stage of development. They are then restricted to the tip link and the accessory structures of the tip link, the upper (UTLD) and lower (LTLD) tip link densities, in mature hair cells (Figure 4) (Kussel-Andermann et al., 2000; Senften et al., 2006; Lefevre et al., 2008; Grillet et al., 2009; Bahloul et al., 2010; Caberlotto et al., 2011; Grati and Kachar, 2011). USH2 proteins are localized at the ankle links of the stereocilia (McGee et al., 2006; van Wijk et al., 2006; Michalski et al., 2007; Yang et al., 2010), which is a transient structure existing only during development (Goodyear et al., 2005). Whirlin is also present at the tip of stereocilia in the vestibular and cochlear hair cells all the time (Belyantseva et al., 2005; Delprat et al., 2005; Kikkawa et al., 2005). Clarin-1 was found at the stereocilia on postnatal day 0 (Zallocchi et al., 2009). Besides their location at the stereocilia, some USH proteins were found at the synaptic region of the outer and inner hair cells (Reiners et al., 2005b; van Wijk et al., 2006; Zallocchi et al., 2009), the cell body of the spinal ganglia (Alagramam et al., 2001b; Adato et al., 2002; van Wijk et al., 2006), the supporting cells (Alagramam et al., 2001b; Adato et al., 2005a; Adato et al., 2005b), various nervous fibers (van Wijk et al., 2006), and Reissner's membrane (Wilson et al., 2001; Lagziel et al., 2005). However, these distributions of USH proteins need to be further verified, because the specificity of antibodies used in the studies were not confirmed in their corresponding mutant mice.

4.2 USH proteins in the retina

In the retina, USH proteins are mainly localized in the photoreceptors (Kremer et al., 2006; Reiners et al., 2006; van Wijk et al., 2006; Liu et al., 2007; Maerker et al., 2008; Yang et al., 2010). The photoreceptor is a highly polarized sensory neuron converting light signals to electrical impulses. It consists of the outer segment, connecting cilium, inner segment, cell body, and synaptic terminus (Figure 4). It contacts Muller cells at the adherens junction (the outer limiting membrane in the retina). Its outer segment is immediately next to the retinal pigment epithelium (RPE) cells.

Compared with the studies in the inner ear, the cellular location of USH proteins is less well defined in the retina. All the USH proteins were once localized in the synaptic ends of photoreceptors (Reiners et al., 2005a; Reiners et al., 2005b; Maerker et al., 2008). However, these results are not conclusive (Williams, 2008; Saihan et al., 2009). They are not supported by the phenotypic analyses in USH mutant mice and the symptom manifestation in USH patients. For instance, ultrastructural abnormalities were not found at the synaptic terminus of photoreceptors in USH mice by electron microscopy (Self et al., 1998; Williams et al., 2009; Yang et al., 2010). No defective ERG waveforms typically resulting from abnormal photoreceptor synaptic transmission have been detected in USH mutant mice (Libby and Steel, 2001; Ball et al., 2003; Libby et al., 2003; Haywood-Watson et al., 2006; McGee et al., 2006; Liu et al., 2007; Yang et al., 2010) or in USH patients.

In addition to the synaptic distribution, MYO7A and SANS were shown to be present around the connecting cilium, harmonin at the outer segment, CDH23 in the inner segment,

and PCDH15 at the base of the outer segment by one research group (Ahmed et al., 2003; Reiners et al., 2005a; Maerker et al., 2008). However, other research groups did not find harmonin in the outer segment (Williams et al., 2009), and MYO7A was demonstrated to be predominantly expressed in the RPE cells (Hasson et al., 1995; el-Amraoui et al., 1996; Lopes et al., 2011). USH2 proteins were initially localized to the inner segment, adherens junction, connecting cilium, basal bodies, and synaptic terminus in photoreceptors (Figure 4) (Kremer et al., 2006; Reiners et al., 2006; van Wijk et al., 2006; Maerker et al., 2008; Lagziel et al., 2009). With the antibodies whose specificities have been confirmed in their respective mutant mice, the three USH2 proteins were recently localized to the periciliary membrane complex (PMC) around the connecting cilium (Figure 4) (Liu et al., 2007; Yang et al., 2010; Yang et al., 2011; Zou et al., 2011). Finally, the distribution of clarin-1 in the retina is controversial. One report shows that it is present around the connecting cilium in photoreceptors (Zallocchi et al., 2009), while the other indicates that clarin-1 is restricted to the Muller cells but not photoreceptors (Geller et al., 2009).

The calycal processes in photoreceptors are thought as an analogous structure to the stereocilia in hair cells (Goodyear and Richardson, 1999). They are well developed in humans, frogs and other species. In mice, only cone photoreceptors have obvious calycal processes (Cohen, 1965; Fetter and Corless, 1987; Rana and Taraszka, 1991). GPR98 and CDH23 are localized at the calycal processes in mouse cone photoreceptors, while whirlin is not evident at this structure in frog photoreceptors (Goodyear and Richardson, 1999; Yang et al., 2010).

5. The USH protein complexes

The indistinguishable symptoms within the same USH clinical type and the similar symptoms across different USH clinical types indicate that various USH proteins probably participate in the same cellular pathway in a broad sense. Among the USH proteins, harmonin, whirlin and SANS possess multiple protein-protein interaction domains and are proposed to be scaffold proteins in multi-protein complexes. Biochemical assays have indeed revealed the existence of their self-interactions and interactions with most of other USH proteins in vitro (Table 2). Interestingly, the in vitro interactions among different USH1 and/or USH2 proteins exist extensively (Table 2). One USH protein is generally able to interact with at least three other USH proteins. In most cases, different regions of the same protein are involved in its binding to different USH proteins (Table 2). Although these interactions have not been individually confirmed in vivo, harmonin, MYO7A, and CDH23 were recently reported to form a ternary complex in hair cells (Bahloul et al., 2010). Based on these findings, it has been hypothesized that USH proteins form an interacting network, an interactome, in both hair cells and photoreceptors (Richardson et al.; Kremer et al., 2006; Reiners et al., 2006; Saihan et al., 2009; Millan et al., 2011).

The above hypothesis is supported by the facts that ablation of one USH protein in mice causes mislocation and/or disappearance of at least one other USH protein in hair cells (Table 3). This phenomenon occurs across USH1 and USH2 proteins. Normal distribution of the three USH2 proteins depends on MYO7A and the distribution of some CDH23 isoform at the tip of the stereocilia relies on GPR98 (Table 3). However, the USH1 and USH2 proteins are present at the different interstereociliary links in hair cells during development. Additionally, different USH proteins are localized at two distinct subcellular locations in

photoreceptors, the PMC and the synapse. Due to these different cellular locations of USH proteins, it is reasonable to propose that more than one USH protein complex exist and they play different but highly related roles in a broad cellular process (Williams, 2008; Yang et al., 2011).

Proteins/domains	Interacting proteins/domains	References
MYO7A		
MyTH4-FERM	Harmonin/PDZ1	(Boeda et al., 2002)
Tail	CDH23/not determined	(Bahloul et al., 2010)
SH2	PCDH15	(Senften et al., 2006)
MyTH4-FERM	SANS/cen	(Wu et al., 2011; Adato et al., 2005b)
MyTH4-FERM	USH2A/cytoplasmic region	(Michalski et al., 2007)
MyTH4-FERM	GPR98/cytoplasmic region	(Michalski et al., 2007)
Not determined	Whirlin/not determined	(Delprat et al., 2005)
Harmonin		
PDZ1	MYO7A/MyTH4-FERM	(Boeda et al., 2002)
N-terminus, PDZ1/2	CDH23/PBMs	(Boeda et al., 2002; Siemens et al., 2002; Grillet et al., 2009; Pan et al., 2009; Bahloul et al., 2010)
PDZ2	PCDH15/CD1 PBM	(Adato et al., 2005b; Reiners et al., 2005b; Senften et al., 2006)
PDZ1/3	SANS/SAM, PBM	(Adato et al., 2005b; Yan et al., 2010)
PDZ1	USH2A/PBM	(Reiners et al., 2005b)
PDZ1	GPR98/PBM	(Reiners et al., 2005b)
PDZ1/2, CC2	Harmonin/PBM, CC2	(Siemens et al., 2002; Adato et al., 2005b)
CDH23		
not determined	MYO7A/tail	(Bahloul et al., 2010)
2 PBMs	Harmonin/N-terminus, PDZ1, PDZ2	(Boeda et al., 2002; Siemens et al., 2002; Grillet et al., 2009; Pan et al., 2009; Bahloul et al., 2010)
EC1-3	PCDH15/EC1	(Kazmierczak et al., 2007)
Cytoplasmic region	SANS/not determined	(Caberlotto et al., 2011)
ECs	CDH23/ECs	(Siemens et al., 2004; Kazmierczak et al., 2007)
PCDH15		
Cytoplasmic region	MYO7A/SH2	(Senften et al., 2006)
CD1 PBM	Harmonin/PDZ2	(Adato et al., 2005b; Reiners et al., 2005b; Senften et al., 2006)
EC1	CDH23/EC1-3	(Kazmierczak et al., 2007)
CD2/CD3	SANS/not determined	(Caberlotto et al., 2011)
ECs	PCDH15/ECs	(Kazmierczak et al., 2007)

Proteins/domains	Interacting proteins/domains	References
SANS		
cen	MYO7A/MyTH4-FERM	(Wu et al, 2011.; Adato et al., 2005b)
SAM, PBM	Harmonin/PDZ1	(Weil et al., 2003; Yan et al., 2010)
Not determined	CDH23/cytoplasmic region	(Caberlotto et al., 2011)
Not determined	PCDH15/CD2, CD3	(Caberlotto et al., 2011)
PBM	Whirlin/PDZ1-PDZ2	(Maerker et al., 2008)
cen	SANS/cen	(Adato et al., 2005b)
USH2A		
Cytoplasmic region	MYO7A/MyTH4-FERM	(Michalski et al., 2007)
PBM	Harmonin/PDZ1	(Reiners et al., 2005b)
PBM	Whirlin/PDZ1-PDZ2	(Adato et al., 2005a; van Wijk et al., 2006; Yang et al., 2010)
GPR98		
Cytoplasmic region	MYO7A/MyTH4-FERM	(Michalski et al., 2007)
PBM	Harmonin/PDZ1	(Reiners et al., 2005b)
PBM	Whirlin/PDZ1-PDZ2	(Adato et al., 2005a; van Wijk et al., 2006; Yang et al., 2010)
Whirlin		
Not determined	MYO7A/not determined	(Delprat et al., 2005)
PDZ1-PDZ2	SANS/PBM	(Maerker et al., 2008)
PDZ1-PDZ2	USH2A/PBM	(Adato et al., 2005a; van Wijk et al., 2006; Yang et al., 2010)
PDZ1-PDZ2	GPR98/PBM	(Adato et al., 2005a; van Wijk et al., 2006; Yang et al., 2010)
PDZ1-PDZ2, PR-PDZ3	Whirlin/PDZ1-PDZ2, PR-PDZ3	(Delprat et al., 2005; Yang et al., 2010)

Table 2. Interactions among USH proteins

In hair cells, the normal cellular localization of harmonin requires the presence of all other USH1 proteins, and loss of harmonin seems not to affect the localization of other USH1 proteins (Table 3), indicating that harmonin is dispensable for locating these USH1 proteins to their normal position in cells. In contrast, CDH23 is relatively independent on other USH1 proteins, and its loss results in mislocalization of the two putative scaffold proteins, harmonin and SANS (Table 3). Therefore, CDH23 may play a crucial role in anchoring/tethering USH1 proteins. Harmonin and SANS may help hold the USH1 proteins in the complex.

Besides the known USH proteins, many other putative components in the USH complexes has been identified. These components are able to interact with at least one of the USH proteins as shown by biochemical assays. For the currently known USH2-interacting proteins, please see the review (Yang et al., 2011). However, additional experiments are necessary to verify the existence of these putative components in the USH complexes in vivo and reveal their relationship with USH.

	MYO7A	USH1C	CDH23	PCDH15	SANS	USH2A
<i>Myo7a</i> ^{-/-}		+(Boeda et al., 2002; Lefevre et al., 2008)	-(Boeda et al., 2002; Senften et al., 2006)	+(Senften et al., 2006)	-(Caberlotto et al., 2011)	+(Michalski et al., 2007)
<i>Ush1c</i> ^{-/-}	+/- (Lefevre et al., 2008; Yan et al., 2011)		-(Lefevre et al., 2008)	+/- (Lefevre et al., 2008; Yan et al., 2011)	+/- (Caberlotto et al., 2011; Yan et al., 2011)	
<i>Cdh23</i> ^{-/-}	+(Bahloul et al., 2010)	+(Lefevre et al., 2008; Bahloul et al., 2010)		-(Senften et al., 2006)	+(Caberlotto et al., 2011)	
<i>Pcdh15</i> ^{-/-}	+(Senften et al., 2006)	+(Lefevre et al., 2008)	-(Senften et al., 2006)		+(Caberlotto et al., 2011)	
<i>Sans</i> ^{-/-}		+(Lefevre et al., 2008)				
<i>Ush2a</i> ^{-/-}						
<i>Gpr98</i> ^{-/-}			+(Michalski et al., 2007)			+(Michalski et al., 2007)
<i>Whrn</i> ^{-/-}						+(Michalski et al., 2007; Yang et al., 2010)

+: existence of mislocalization, -: normal localization, +/-, contradictory results

Table 3. Interdependence of USH proteins in hair cells

6. Functions of the USH complexes

The severe and early-onset hearing phenotypes in various USH1 and USH2 mouse models make it relatively easier to decipher the functions of USH complexes in the inner ear than in the retina. The following will focus on the three main cellular processes generally believed to involve the USH complexes. Disruption of these USH functions is thought to be the molecular mechanisms underlying USH.

6.1 Hair bundle cohesion

During development, at the apex of hair cells, microvilli grow into stereocilia by recruiting more actin filaments. These stereocilia are bundled with transient lateral links and are connected with the kinocilium through kinociliary links. Following the establishment of the planar cell polarity, the kinocilium moves from the center to the periphery of the cell, and the stereocilia elongate differentially. The staircase-shape hair bundle is eventually formed. At the same time, the transient lateral links are gradually substituted by two distinct sets of interstereociliary links. They are the horizontal top connectors and the ankle links, close to the tip and base of the hair bundle, respectively (Figure 4). The tip links emerge, which are fibrous connections between the tip of medium and low stereocilia and the side of the neighboring taller stereocilia (Figure 4). Finally, the stereocilia grow both in length and in width and reach their mature size. In rodent mature cochlear hair cells, the ankle links and the kinociliary links disappear with the regression of the kinocilium (Frolenkov et al., 2004; Goodyear et al., 2005; Nayak et al., 2007).

CDH23 (Siemens et al., 2004; Lagziel et al., 2005; Michel et al., 2005; Rzadzinska et al., 2005; Lefevre et al., 2008) and PCDH15 (Goodyear et al., 2010; Webb et al., 2011; Lefevre et al., 2008) are localized at the transient lateral links and kinociliary links during early development of hair cells. In their mutant mice, hair bundles are usually splayed into several clumps; kinocilium is mispositioned and disconnected with the hair bundle (Lefevre et al., 2008), indicating that CDH23 and PCDH15, as components of the interstereociliary links, are important for hair bundle cohesion and that loss of the connection between the stereocilia and kinocilium causes the misorientation of the hair bundle. Interestingly, the mutant mouse models of all five USH1 genes share such similar phenotypes. This could be explained by the idea that the five USH1 proteins coordinate in this function. The PST domain of harmonin b binds to and bundles actin filaments (Boeda et al., 2002). MYO7A is a high duty ratio motor, which binds to actin filament strongly. Therefore, these two actin-binding proteins may anchor their interacting partners, CDH23 and PCDH15, to the actin bundle in the stereocilia of hair cells (Table 2). In *Ush1g*^{-/-} mice, cohesion of stereocilia is disrupted. In *Ush1g*^{fl/fl}*Myo7a-cre*^{+/-} mice, whose expression of SANS is disturbed only after birth, the stereocilia stay cohesive (Caberlotto et al., 2011). Therefore, SANS plays a role in stereocilia cohesion during the prenatal period. It may be involved in the organization of other USH1 proteins through directly interacting with them (Table 2).

All three USH2 proteins, USH2A, GPR98, and whirlin, are positioned at the ankle links of hair cells. Among these proteins, USH2A and GPR98 probably interact with each other or with some unidentified cell adhesion proteins to form the ankle links. Whirlin interacts with USH2A and GPR98 through the PDZ domain-mediated binding to anchor them at the base of the stereocilia. In the absence of GPR98, the ankle links are missing. Thus far, the

dependence of the ankle links on USH2A and whirlin has not been examined. In the wild-type mouse, the stereocilia of outer hair cells are organized into a V-shaped staircase-like hair bundle. However, in all three *Ush2* mutant mice, the outer hair cells show various disorganized stereocilia and abnormal U-shape hair bundles (Mburu et al., 2003; McGee et al., 2006; Liu et al., 2007; Michalski et al., 2007; Yang et al., 2010). Accordingly, as components of the ankle links, the three USH2 proteins probably contribute to hair bundle cohesion as well.

6.2 Mechanotransduction

The stereocilia of hair cells are the cellular organelle conducting mechanotransduction. The vibration of the basilar membrane and tectorial membrane or the motion of endolymphatic fluid induces the hair bundle deflection. When the deflection is toward the longest stereocilia (the positive or excitatory direction), the transduction channels are open. The influx of Ca^{2+} and K^{+} through the channels elicits changes of the membrane potential and glutamate release at the ribbon synapse in hair cells. When the hair bundle moves away from the longest stereocilia (the negative or inhibitory direction), the transduction channels close, and the membrane potential and transmitter release resume their resting statuses. Although the molecular machinery of mechanotransduction is not well understood, the 'gating spring' model is popular in the field. In this model, the tip link, whose axis is parallel to the direction of the mechanical sensitivity of the hair bundle, is thought as a sensor to the stretch of the hair bundle. Alternatively, an unknown structure attached to the tip link fulfills this function (Vollrath et al., 2007; Gillespie and Muller, 2009). The transduction channel was recently localized to the plasma membrane at the lower end of the tip link in the stereocilia (Beurg et al., 2008).

In mature hair cells, CDH23 (Siemens et al., 2004; Sollner et al., 2004) and PCDH15 (Ahmed et al., 2006) were found associated with the tip links. CDH23 is mainly at the upper part and PCDH15 at the lower part of the links (Kazmierczak et al., 2007; Alagramam et al., 2011). In *Cdh23^{V-2J}* and *Pcdh15^{aw-6J}* mice, the tip links are missing. Additionally, the response of the mechanotransduction is reduced. In the absence of stimulus, a fraction of transduction channels keep open in the wild-type hair cells, due to the resting tension of the tip links. However, the transduction channels in these two mutants do not open or take up the styryl dye FM1-43 at rest (Senften et al., 2006; Alagramam et al., 2011). Therefore, CDH23 and PCDH15 are believed to be components of the tip links and to participate in mechanotransduction in mature hair cells.

At the two ends of the tip link immediately beneath the stereocilia plasma membrane, there are electron-dense complexes, the UTLD and LTLD (Figure 4). Harmonin and MYO7A are present at the UTLD (Grillet et al., 2009; Michalski et al., 2009; Caberlotto et al., 2011; Grati and Kachar, 2011). In *Myo7a^{6J}*, *Myo7a^{4626SB}*, *Ush1c^{dfcr}*, and *Ush1c^{dfcr-2J}* mice, the adaptation of mechanotransduction, a process for the hair cells to recover their sensitivity under sustained mechanical stimulation, was found consistently abnormal, while the amplitude of mechanotransduction responses is sometimes normal (Kros et al., 2002; Grillet et al., 2009; Michalski et al., 2009). These results suggest that harmonin and MYO7A are involved in the transduction adaptation. SANS may exist at both the LTLD and UTLD (Caberlotto et al., 2011; Grati and Kachar, 2011). Its loss in hair cells (*Ush1g^{-/-}*) causes elimination of the tip links and reduction in both the amplitude and sensitivity of the transduction currents

(Caberlotto et al., 2011). In *Ush1g^{fl/fl} Myo7a-cre^{+/-}* mice, whose hair bundle morphology is intact, only the amplitude of transduction is affected. This finding indicates that SANS is implicated in mechanotransduction and plays a different role from harmonin or MYO7A.

Gpr98 knockout and *Gpr98^{del7TM}* mice also show defects in mechanotransduction, though there are some discrepancies between them (McGee et al., 2006; Michalski et al., 2007). In general, the sensitivity to the stimulation direction is changed in both outer and inner hair cells. The amplitude and sensitivity of the transduction current decrease in the outer hair cells, but are normal in the inner hair cells and the utricular hair cells. It is suggested that the misorganization of hair bundles in *Gpr98* mutant mice accounts of the abnormal sensitivity direction. Alternatively, GPR98 could be indirectly related with the cellular process of mechanotransduction.

6.3 Protein and organelle transport

In photoreceptors, the outer segment is a large specialized cilium filled with many flat membrane disks, where phototransduction occurs (Figure 4). This cellular compartment undergoes continuous and rapid renewal (Young, 1967; LaVail, 1976; Young, 1976; Besharse and Hollyfield, 1979), which requires a large amount of proteins and membrane lipids to be synthesized in the inner segment and to be quickly transported to the base of the outer segment through the connecting cilium (Figure 4). The removal of the old outer segment is achieved through phagocytosis by RPE cells. In addition, in both photoreceptors and RPE cells, several proteins, involved in phototransduction and retinoid cycle, translocate between two different cellular compartments in response to light (Artemyev, 2008; Slepak and Hurley, 2008; Lopes et al., 2011).

Among USH proteins, MYO7A is an actin-based motor. In the retina, it is expressed in both RPE cells and photoreceptors. In RPE cells, MYO7A is essential for the transport of phagosomes to their degradation apparatus (Gibbs et al., 2003), tethering melanosomes during their movement (Gibbs et al., 2004), and the translocation of RPE65 responding to light exposure (Lopes et al., 2011). In photoreceptors, MYO7A is present along the connecting cilium. Loss of MYO7A was found to delay the transport of opsin from the inner to the outer segment (Liu et al., 1999) and the transducin translocation from the outer to the inner segment after light exposure (Peng et al., 2011). In hair cells, without MYO7A, all USH2 proteins are mislocalized from the ankle links (Table 3), suggesting that MYO7A may transport the USH2 proteins. These lines of evidence establish the notion that MYO7A may function in protein and organelle transport in various cells in the retina and the inner ear.

USH2 proteins are positioned at the PMC in mammalian photoreceptors, which is an analogous structure to the periciliary ridge complex (PRC) in frogs (Peters et al., 1983). The PRC is a morphologically-specialized structure with a symmetrical array of 9 ridges and 9 grooves. It has been proposed, based on immunocytochemistry and freeze-fracture electron microscopy, as the membrane fusion site for post-Golgi vesicles carrying opsin and docosahexaenoyl (DHA)-phospholipids before these cargos are transported from the inner to the outer segment (Peters et al., 1983; Papermaster et al., 1986; Rodriguez de Turco et al., 1997; Papermaster, 2002). Additionally, Rab8, rac1, Sec8, moesin, syntaxin 3 and SNAP-25 have been localized around the PRC in frog photoreceptors (Deretic et al., 2004; Mazelova et al., 2009). These proteins are proposed, though not verified using mouse genetics, to

participate in and/or regulate the docking and membrane fusion of post-Golgi vesicles to the plasma membrane at the PRC. Therefore, the USH2 complex at the PMC might play either a direct or indirect role in the docking between the post-Golgi vesicles and plasma membrane at the base of the connecting cilium (Roepman and Wolfrum, 2007; Maerker et al., 2008). This proposed function can also be applied in hair cells. The ankle-links exist when stereocilia grow and differentiate from small microvilli. At this time, many vesicles are at the base of stereocilia (Forge et al., 1997; Hasson et al., 1997), which could be the post-Golgi vesicles carrying proteins and membrane lipids from the cell body to the growing stereocilia. Supportively, the *Gpr98* knockout mouse shows delocalization of some CDH23 long isoforms at the tip of the stereocilia and, possibly, loss of some apical links between the stereocilia (Michalski et al., 2007). However, solid evidence supporting this putative function of the USH2 complex is still scarce. For instance, obvious mislocalization of rhodopsin has not been observed in whirlin knockout and *Ush2a* knockout mice (Liu et al., 2007; Yang et al., 2010), and vesicles fused with the plasma membrane have not been demonstrated at the ankle links.

7. Therapeutic studies

Because of the widespread clinical application of the well-developed cochlear implant for hearing loss (Pennings et al., 2006; Liu et al., 2008), more attention is focused on seeking effective treatments for retinitis pigmentosa in USH. Next, I will address the current progress in studies on gene therapy, drug application, cell transplantation, and nutritional supplements (Yang et al., 2011).

Human neural progenitor cells from the post mortem fetal cortical brain have been tested in the *Ush2a* knockout mouse (Lu et al., 2009). The progenitor cells were transplanted between photoreceptors and RPE cells. There, they delayed the cellular changes in photoreceptors and alleviated retinal functional deterioration. However, due to the short follow-up time after the treatment, the study did not examine whether the treatment can rescue photoreceptor loss in this animal model.

Compared to the cell-based therapy, replacement of the mutant gene in the retina is straightforward. The efficiency and efficacy of a lentivirus-mediated gene replacement of MYO7A have been studied in the *Myo7a*^{4626SB} mouse (Hashimoto et al., 2007). Although the delivery of MYO7A into photoreceptors and RPE cells is not quite efficient, the treated mutant retina does show correction of the histological phenotypes in these two cells. In addition, our laboratory utilized a combination of AAV and a photoreceptor-specific promoter to efficiently target the USH2D gene, whirlin, into both rod and cone photoreceptors. The transgenic whirlin was found to restore the changes of USH2A and GPR98 expression in the whirlin knockout retina (Zou et al., 2011). These encouraging progresses in the USH1B and USH2D mouse models lay a solid foundation for a further and detailed exploration of gene therapy for these and other USH subtypes.

Aminoglycosides and their derivatives can induce a read-through of nonsense mutations by inserting an amino acid at the stop codon. These drugs have been tested in vitro, in cell cultures and in retinal explants to suppress the nonsense mutations found in USH1F (PCDH15) and USH1C (harmonin) patients (Rebibo-Sabbah et al., 2007; Nudelman et al., 2009; Goldmann et al., 2010; Nudelman et al., 2010). However, the high cellular toxicity of

these drugs and the low efficiency of their read-through activities set a hindrance for their further application to patients. A recent report has shown that PTC124, a drug unrelated to aminoglycosides, has a relatively low cellular toxicity and high read-through efficacy (Goldmann et al., 2011).

The nutritional supplementation, daily intakes of vitamin A at a dose of 15,000 international units (IU) and vitamin E less than 400 IU, is thought to be a potential effective therapy for retinitis pigmentosa (Berson et al., 1993; Berson, 2000). Although it has already been applied to patients, this vitamin A supplement therapy is still under debate and its underlying mechanism is unknown.

8. Summary and perspective

The research on USH has made tremendous progress since the discovery of its first causative gene, *MYO7A*, in 1995. Currently, nine genes have been identified responsible for this genetic disease. From the functional domain analysis, these genes have been proposed to participate in trafficking, scaffolding, cell adhesion, and signaling in cells. Many spontaneous and transgenic mouse, rat, and zebrafish models are available now. The majority of these animal models reproduce the hearing and balance problems in USH patients. However, not many of them manifest retinal degeneration, which is one of the typical symptoms in USH patients. The reason for this discrepancy is not clear. But lack of retinal phenotypes in these animal models hinders our studies on retinitis pigmentosa in USH patients. A large body of evidence from biochemical and cellular localization studies demonstrate that USH proteins are organized into multi-component complexes mainly in hair cells and photoreceptors. They play a role in hair bundle cohesion, mechanotransduction, and, possibly, protein/organelle transport in vivo. USH is an incurable disease. Effective treatments using different approaches are still being sought and explored.

9. References

- Aarnisalo AA, Pietola L, Joensuu J, Isosomppi J, Aarnisalo P, Dinculescu A, Lewin AS, Flannery J, Hauswirth WW, Sankila EM, Jero J (2007) Anti-clarin-1 AAV-delivered ribozyme induced apoptosis in the mouse cochlea. *Hear Res* 230:9-16.
- Adato A, Lefevre G, Delprat B, Michel V, Michalski N, Chardenoux S, Weil D, El-Amraoui A, Petit C (2005a) Usherin, the defective protein in Usher syndrome type IIA, is likely to be a component of interstereocilia ankle links in the inner ear sensory cells. *Hum Mol Genet* 14:3921-3932.
- Adato A, Michel V, Kikkawa Y, Reiners J, Alagramam KN, Weil D, Yonekawa H, Wolfrum U, El-Amraoui A, Petit C (2005b) Interactions in the network of Usher syndrome type 1 proteins. *Hum Mol Genet* 14:347-356.
- Adato A, Vreugde S, Joensuu T, Avidan N, Hamalainen R, Belenkiy O, Olender T, Bonne-Tamir B, Ben-Asher E, Espinos C, Millan JM, Lehesjoki AE, Flannery JG, Avraham KB, Pietrokovski S, Sankila EM, Beckmann JS, Lancet D (2002) USH3A transcripts encode clarin-1, a four-transmembrane-domain protein with a possible role in sensory synapses. *Eur J Hum Genet* 10:339-350.

- Ahmed ZM, Riazuddin S, Khan SN, Friedman PL, Friedman TB (2009) USH1H, a novel locus for type I Usher syndrome, maps to chromosome 15q22-23. *Clin Genet* 75:86-91.
- Ahmed ZM, Riazuddin S, Bernstein SL, Ahmed Z, Khan S, Griffith AJ, Morell RJ, Friedman TB, Wilcox ER (2001) Mutations of the protocadherin gene PCDH15 cause Usher syndrome type 1F. *Am J Hum Genet* 69:25-34.
- Ahmed ZM, Smith TN, Riazuddin S, Makishima T, Ghosh M, Bokhari S, Menon PS, Deshmukh D, Griffith AJ, Friedman TB, Wilcox ER (2002) Nonsyndromic recessive deafness DFNB18 and Usher syndrome type IC are allelic mutations of USH1C. *Hum Genet* 110:527-531.
- Ahmed ZM, Riazuddin S, Ahmad J, Bernstein SL, Guo Y, Sabar MF, Sieving P, Griffith AJ, Friedman TB, Belyantseva IA, Wilcox ER (2003) PCDH15 is expressed in the neurosensory epithelium of the eye and ear and mutant alleles are responsible for both USH1F and DFNB23. *Hum Mol Genet* 12:3215-3223.
- Ahmed ZM, Goodyear R, Riazuddin S, Lagziel A, Legan PK, Behra M, Burgess SM, Lilley KS, Wilcox ER, Griffith AJ, Frolenkov GI, Belyantseva IA, Richardson GP, Friedman TB (2006) The tip-link antigen, a protein associated with the transduction complex of sensory hair cells, is protocadherin-15. *J Neurosci* 26:7022-7034.
- Alagramam KN, Murcia CL, Kwon HY, Pawlowski KS, Wright CG, Woychik RP (2001a) The mouse Ames waltzer hearing-loss mutant is caused by mutation of Pcdh15, a novel protocadherin gene. *Nat Genet* 27:99-102.
- Alagramam KN, Goodyear RJ, Geng R, Furness DN, van Aken AF, Marcotti W, Kros CJ, Richardson GP (2011) Mutations in protocadherin 15 and cadherin 23 affect tip links and mechanotransduction in mammalian sensory hair cells. *PLoS One* 6:e19183.
- Alagramam KN, Yuan H, Kuehn MH, Murcia CL, Wayne S, Srisailpathy CR, Lowry RB, Knaus R, Van Laer L, Bernier FP, Schwartz S, Lee C, Morton CC, Mullins RF, Ramesh A, Van Camp G, Hageman GS, Woychik RP, Smith RJ (2001b) Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F. *Hum Mol Genet* 10:1709-1718.
- Aller E, Najera C, Millan JM, Oltra JS, Perez-Garrigues H, Vilela C, Navea A, Beneyto M (2004) Genetic analysis of 2299delG and C759F mutations (USH2A) in patients with visual and/or auditory impairments. *Eur J Hum Genet* 12:407-410.
- Artemyev NO (2008) Light-dependent compartmentalization of transducin in rod photoreceptors. *Mol Neurobiol* 37:44-51.
- Astuto LM, Weston MD, Carney CA, Hoover DM, Cremers CW, Wagenaar M, Moller C, Smith RJ, Pieke-Dahl S, Greenberg J, Ramesar R, Jacobson SG, Ayuso C, Heckenlively JR, Tamayo M, Gorin MB, Reardon W, Kimberling WJ (2000) Genetic heterogeneity of Usher syndrome: analysis of 151 families with Usher type I. *Am J Hum Genet* 67:1569-1574.
- Audo I, Bujakowska K, Mohand-Said S, Tronche S, Lancelot ME, Antonio A, Germain A, Lonjou C, Carpentier W, Sahel JA, Bhattacharya S, Zeitze C (2011) A novel DFNB31 mutation associated with Usher type 2 syndrome showing variable degrees of auditory loss in a consanguineous Portuguese family. *Mol Vis* 17:1598-1606.
- Bahloul A, Michel V, Hardelin JP, Nouaille S, Hoos S, Houdusse A, England P, Petit C (2010) Cadherin-23, myosin VIIa and harmonin, encoded by Usher syndrome type I

- genes, form a ternary complex and interact with membrane phospholipids. *Hum Mol Genet* 19:3557-3565.
- Ball SL, Bardenstein D, Alagramam KN (2003) Assessment of retinal structure and function in Ames waltzer mice. *Invest Ophthalmol Vis Sci* 44:3986-3992.
- Bashir R, Fatima A, Naz S (2011) A frameshift mutation in SANS results in atypical Usher syndrome. *Clin Genet* 78:601-603.
- Baux D, Larrieu L, Blanchet C, Hamel C, Ben Salah S, Vielle A, Gilbert-Dussardier B, Holder M, Calvas P, Philip N, Edery P, Bonneau D, Claustres M, Malcolm S, Roux AF (2007) Molecular and in silico analyses of the full-length isoform of usherin identify new pathogenic alleles in Usher type II patients. *Hum Mutat* 28:781-789.
- Belyantseva IA, Boger ET, Naz S, Frolenkov GI, Sellers JR, Ahmed ZM, Griffith AJ, Friedman TB (2005) Myosin-XVa is required for tip localization of whirlin and differential elongation of hair-cell stereocilia. *Nat Cell Biol* 7:148-156.
- Ben Rebeh I, Benzina Z, Dhouib H, Hadjamor I, Amyere M, Ayadi L, Turki K, Hammami B, Kmiha N, Kammoun H, Hakim B, Charfedine I, Vikkula M, Ghorbel A, Ayadi H, Masmoudi S (2008) Identification of candidate regions for a novel Usher syndrome type II locus. *Mol Vis* 14:1719-1726.
- Berson EL (2000) Nutrition and retinal degenerations. *Int Ophthalmol Clin* 40:93-111.
- Berson EL, Rosner B, Sandberg MA, Hayes KC, Nicholson BW, Weigel-DiFranco C, Willett W (1993) A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. *Arch Ophthalmol* 111:761-772.
- Besharse JC, Hollyfield JG (1979) Turnover of mouse photoreceptor outer segments in constant light and darkness. *Invest Ophthalmol Vis Sci* 18:1019-1024.
- Beurg M, Nam JH, Crawford A, Fettiplace R (2008) The actions of calcium on hair bundle mechanics in mammalian cochlear hair cells. *Biophys J* 94:2639-2653.
- Bhattacharya G, Miller C, Kimberling WJ, Jablonski MM, Cosgrove D (2002) Localization and expression of usherin: a novel basement membrane protein defective in people with Usher's syndrome type IIa. *Hear Res* 163:1-11.
- Bitner-Glindzicz M, Lindley KJ, Rutland P, Blaydon D, Smith VV, Milla PJ, Hussain K, Furth-Lavi J, Cosgrove KE, Shepherd RM, Barnes PD, O'Brien RE, Farndon PA, Sowden J, Liu XZ, Scanlan MJ, Malcolm S, Dunne MJ, Aynsley-Green A, Glaser B (2000) A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene. *Nat Genet* 26:56-60.
- Boeda B, El-Amraoui A, Bahloul A, Goodyear R, Daviet L, Blanchard S, Perfettini I, Fath KR, Shorte S, Reiners J, Houdusse A, Legrain P, Wolfrum U, Richardson G, Petit C (2002) Myosin VIIa, harmonin and cadherin 23, three Usher I gene products that cooperate to shape the sensory hair cell bundle. *Embo J* 21:6689-6699.
- Bok D, Galbraith G, Lopez I, Woodruff M, Nusinowitz S, BeltrandelRio H, Huang W, Zhao S, Geske R, Montgomery C, Van Sligtenhorst I, Friddle C, Platt K, Sparks MJ, Pushkin A, Abuladze N, Ishiyama A, Dukkupati R, Liu W, Kurtz I (2003) Blindness and auditory impairment caused by loss of the sodium bicarbonate cotransporter NBC3. *Nat Genet* 34:313-319.
- Bolz H, von Brederlow B, Ramirez A, Bryda EC, Kutsche K, Nothwang HG, Seeliger M, del CSCM, Vila MC, Molina OP, Gal A, Kubisch C (2001) Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. *Nat Genet* 27:108-112.

- Bork JM et al. (2001) Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. *Am J Hum Genet* 68:26-37.
- Boughman JA, Vernon M, Shaver KA (1983) Usher syndrome: definition and estimate of prevalence from two high-risk populations. *J Chronic Dis* 36:595-603.
- Caberlotto E, Michel V, Foucher I, Bahloul A, Goodyear RJ, Pepermans E, Michalski N, Perfettini I, Alegria-Prevot O, Chardenoux S, Do Cruzeiro M, Hardelin JP, Richardson GP, Avan P, Weil D, Petit C (2011) Usher type 1G protein sans is a critical component of the tip-link complex, a structure controlling actin polymerization in stereocilia. *Proc Natl Acad Sci U S A* 108:5825-5830.
- Chaib H, Kaplan J, Gerber S, Vincent C, Ayadi H, Slim R, Munnich A, Weissenbach J, Petit C (1997) A newly identified locus for Usher syndrome type I, USH1E, maps to chromosome 21q21. *Hum Mol Genet* 6:27-31.
- Cohen AI (1965) New Details of the Ultrastructure of the Outer Segments and Ciliary Connectives of the Rods of Human and Macaque Retinas. *Anat Rec* 152:63-79.
- Cohen M, Bitner-Glindzicz M, Luxon L (2007) The changing face of Usher syndrome: clinical implications. *Int J Audiol* 46:82-93.
- Delprat B, Michel V, Goodyear R, Yamasaki Y, Michalski N, El-Amraoui A, Perfettini I, Legrain P, Richardson G, Hardelin JP, Petit C (2005) Myosin XVa and whirlin, two deafness gene products required for hair bundle growth, are located at the stereocilia tips and interact directly. *Hum Mol Genet* 14:401-410.
- Deretic D, Traverso V, Parkins N, Jackson F, Rodriguez de Turco EB, Ransom N (2004) Phosphoinositides, ezrin/moesin, and rac1 regulate fusion of rhodopsin transport carriers in retinal photoreceptors. *Mol Biol Cell* 15:359-370.
- Di Palma F, Pellegrino R, Noben-Trauth K (2001a) Genomic structure, alternative splice forms and normal and mutant alleles of cadherin 23 (*Cdh23*). *Gene* 281:31-41.
- Di Palma F, Holme RH, Bryda EC, Belyantseva IA, Pellegrino R, Kachar B, Steel KP, Noben-Trauth K (2001b) Mutations in *Cdh23*, encoding a new type of cadherin, cause stereocilia disorganization in waltzer, the mouse model for Usher syndrome type 1D. *Nat Genet* 27:103-107.
- Dreyer B, Tranebjaerg L, Rosenberg T, Weston MD, Kimberling WJ, Nilssen O (2000) Identification of novel USH2A mutations: implications for the structure of USH2A protein. *Eur J Hum Genet* 8:500-506.
- Dreyer B, Brox V, Tranebjaerg L, Rosenberg T, Sadeghi AM, Moller C, Nilssen O (2008) Spectrum of USH2A mutations in Scandinavian patients with Usher syndrome type II. *Hum Mutat* 29:451.
- Ebermann I, Wiesen MH, Zrenner E, Lopez I, Pigeon R, Kohl S, Lowenheim H, Koenekoop RK, Bolz HJ (2009) GPR98 mutations cause Usher syndrome type 2 in males. *J Med Genet* 46:277-280.
- Ebermann I, Scholl HP, Charbel Issa P, Becirovic E, Lamprecht J, Jurklies B, Millan JM, Aller E, Mitter D, Bolz H (2006) A novel gene for Usher syndrome type 2: mutations in the long isoform of whirlin are associated with retinitis pigmentosa and sensorineural hearing loss. *Hum Genet* 121:203-211.
- Ebermann I, Phillips JB, Liebau MC, Koenekoop RK, Schermer B, Lopez I, Schafer E, Roux AF, Dafinger C, Bernd A, Zrenner E, Claustres M, Blanco B, Nurnberg G, Nurnberg P, Ruland R, Westerfield M, Benzing T, Bolz HJ (2010) PDZD7 is a modifier of

- retinal disease and a contributor to digenic Usher syndrome. *J Clin Invest* 120:1812-1823.
- Edwards A, Fishman GA, Anderson RJ, Grover S, Derlacki DJ (1998) Visual acuity and visual field impairment in Usher syndrome. *Arch Ophthalmol* 116:165-168.
- El-Amraoui A, Petit C (2005) Usher I syndrome: unravelling the mechanisms that underlie the cohesion of the growing hair bundle in inner ear sensory cells. *J Cell Sci* 118:4593-4603.
- El-Amraoui A, Petit C (2010) Cadherins as targets for genetic diseases. *Cold Spring Harb Perspect Biol* 2:a003095.
- el-Amraoui A, Sahly I, Picaud S, Sahel J, Abitbol M, Petit C (1996) Human Usher 1B/mouse shaker-1: the retinal phenotype discrepancy explained by the presence/absence of myosin VIIA in the photoreceptor cells. *Hum Mol Genet* 5:1171-1178.
- Ernest S, Rauch GJ, Haffter P, Geisler R, Petit C, Nicolson T (2000) Mariner is defective in myosin VIIA: a zebrafish model for human hereditary deafness. *Hum Mol Genet* 9:2189-2196.
- Eudy JD, Weston MD, Yao S, Hoover DM, Rehm HL, Ma-Edmonds M, Yan D, Ahmad I, Cheng JJ, Ayuso C, Cremers C, Davenport S, Moller C, Talmadge CB, Beisel KW, Tamayo M, Morton CC, Swaroop A, Kimberling WJ, Sumegi J (1998) Mutation of a gene encoding a protein with extracellular matrix motifs in Usher syndrome type IIa. *Science* 280:1753-1757.
- Fetter RD, Corless JM (1987) Morphological components associated with frog cone outer segment disc margins. *Invest Ophthalmol Vis Sci* 28:646-657.
- Fields RR, Zhou G, Huang D, Davis JR, Moller C, Jacobson SG, Kimberling WJ, Sumegi J (2002) Usher syndrome type III: revised genomic structure of the USH3 gene and identification of novel mutations. *Am J Hum Genet* 71:607-617.
- Fishman GA, Bozbeyoglu S, Massof RW, Kimberling W (2007) Natural course of visual field loss in patients with Type 2 Usher syndrome. *Retina* 27:601-608.
- Forge A, Souter M, Denman-Johnson K (1997) Structural development of sensory cells in the ear. *Semin Cell Dev Biol* 8:225-237.
- Frolenkov GI, Belyantseva IA, Friedman TB, Griffith AJ (2004) Genetic insights into the morphogenesis of inner ear hair cells. *Nat Rev Genet* 5:489-498.
- Geller SF, Guerin KI, Visel M, Pham A, Lee ES, Dror AA, Avraham KB, Hayashi T, Ray CA, Reh TA, Birmingham-McDonogh O, Triffo WJ, Bao S, Isosomppi J, Västinsalo H, Sankila EM, Flannery JG (2009) CLRN1 is nonessential in the mouse retina but is required for cochlear hair cell development. *PLoS Genet* 5:e1000607.
- Geng R, Geller SF, Hayashi T, Ray CA, Reh TA, Birmingham-McDonogh O, Jones SM, Wright CG, Melki S, Imanishi Y, Palczewski K, Alagramam KN, Flannery JG (2009) Usher syndrome IIIA gene clarin-1 is essential for hair cell function and associated neural activation. *Hum Mol Genet* 18:2748-2760.
- Gerber S, Bonneau D, Gilbert B, Munnich A, Dufier JL, Rozet JM, Kaplan J (2006) USH1A: chronicle of a slow death. *Am J Hum Genet* 78:357-359.
- Gibbs D, Kitamoto J, Williams DS (2003) Abnormal phagocytosis by retinal pigmented epithelium that lacks myosin VIIa, the Usher syndrome 1B protein. *Proc Natl Acad Sci U S A* 100:6481-6486.

- Gibbs D, Azarian SM, Lillo C, Kitamoto J, Klomp AE, Steel KP, Libby RT, Williams DS (2004) Role of myosin VIIa and Rab27a in the motility and localization of RPE melanosomes. *J Cell Sci* 117:6473-6483.
- Gibert Y, McMillan DR, Kayes-Wandover K, Meyer A, Begemann G, White PC (2005) Analysis of the very large G-protein coupled receptor gene (Vlgr1/Mass1/USH2C) in zebrafish. *Gene* 353:200-206.
- Gillespie PG, Muller U (2009) Mechanotransduction by hair cells: models, molecules, and mechanisms. *Cell* 139:33-44.
- Goldmann T, Overlack N, Wolfrum U, Nagel-Wolfrum K (2011) PTC124 mediated translational read-through of a nonsense mutation causing Usher type 1C. *Hum Gene Ther* 22:537-547.
- Goldmann T, Rebibo-Sabbah A, Overlack N, Nudelman I, Belakhov V, Baasov T, Ben-Yosef T, Wolfrum U, Nagel-Wolfrum K (2010) Beneficial read-through of a USH1C nonsense mutation by designed aminoglycoside NB30 in the retina. *Invest Ophthalmol Vis Sci* 51:6671-6680.
- Goodyear R, Richardson G (1999) The ankle-link antigen: an epitope sensitive to calcium chelation associated with the hair-cell surface and the calycal processes of photoreceptors. *J Neurosci* 19:3761-3772.
- Goodyear RJ, Richardson GP (2003) A novel antigen sensitive to calcium chelation that is associated with the tip links and kinocilial links of sensory hair bundles. *J Neurosci* 23:4878-4887.
- Goodyear RJ, Forge A, Legan PK, Richardson GP (2010) Asymmetric distribution of cadherin 23 and protocadherin 15 in the kinocilial links of avian sensory hair cells. *J Comp Neurol* 518:4288-4297.
- Goodyear RJ, Marcotti W, Kros CJ, Richardson GP (2005) Development and properties of stereociliary link types in hair cells of the mouse cochlea. *J Comp Neurol* 485:75-85.
- Grati M, Kachar B (2011) Myosin VIIa and sans localization at stereocilia upper tip-link density implicates these Usher syndrome proteins in mechanotransduction. *Proc Natl Acad Sci U S A* 108:11476-11481.
- Grillet N, Xiong W, Reynolds A, Kazmierczak P, Sato T, Lillo C, Dumont RA, Hintermann E, Sczaniecka A, Schwander M, Williams D, Kachar B, Gillespie PG, Muller U (2009) Harmonin mutations cause mechanotransduction defects in cochlear hair cells. *Neuron* 62:375-387.
- Grondahl J (1987) Estimation of prognosis and prevalence of retinitis pigmentosa and Usher syndrome in Norway. *Clin Genet* 31:255-264.
- Hampton LL, Wright CG, Alagramam KN, Battey JF, Noben-Trauth K (2003) A new spontaneous mutation in the mouse Ames waltzer gene, *Pcdh15*. *Hear Res* 180:67-75.
- Han F, Yu H, Tian C, Chen HE, Benedict-Alderfer C, Zheng Y, Wang Q, Han X, Zheng QY (2010) A new mouse mutant of the *Cdh23* gene with early-onset hearing loss facilitates evaluation of otoprotection drugs. *Pharmacogenomics J*.
- Hartong DT, Berson EL, Dryja TP (2006) Retinitis pigmentosa. *Lancet* 368:1795-1809.
- Hashimoto T, Gibbs D, Lillo C, Azarian SM, Legacki E, Zhang XM, Yang XJ, Williams DS (2007) Lentiviral gene replacement therapy of retinas in a mouse model for Usher syndrome type 1B. *Gene Ther* 14:584-594.

- Hasson T, Heintzelman MB, Santos-Sacchi J, Corey DP, Mooseker MS (1995) Expression in cochlea and retina of myosin VIIa, the gene product defective in Usher syndrome type 1B. *Proc Natl Acad Sci U S A* 92:9815-9819.
- Hasson T, Walsh J, Cable J, Mooseker MS, Brown SD, Steel KP (1997) Effects of shaker-1 mutations on myosin-VIIa protein and mRNA expression. *Cell Motil Cytoskeleton* 37:127-138.
- Haywood-Watson RJ, 2nd, Ahmed ZM, Kjellstrom S, Bush RA, Takada Y, Hampton LL, Battey JF, Sieving PA, Friedman TB (2006) Ames Waltzer deaf mice have reduced electroretinogram amplitudes and complex alternative splicing of Pcdh15 transcripts. *Invest Ophthalmol Vis Sci* 47:3074-3084.
- Heissler SM, Manstein DJ (2011) Functional characterization of the human myosin-7a motor domain. *Cell Mol Life Sci*.
- Hilgert N, Kahrizi K, Dieltjens N, Bazazzadegan N, Najmabadi H, Smith RJ, Van Camp G (2009) A large deletion in GPR98 causes type IIC Usher syndrome in male and female members of an Iranian family. *J Med Genet* 46:272-276.
- Hmani-Aifa M, Benzina Z, Zulfiqar F, Dhouib H, Shahzadi A, Ghorbel A, Rebai A, Soderkvist P, Riazuddin S, Kimberling WJ, Ayadi H (2009) Identification of two new mutations in the GPR98 and the PDE6B genes segregating in a Tunisian family. *Eur J Hum Genet* 17:474-482.
- Holme RH, Kiernan BW, Brown SD, Steel KP (2002) Elongation of hair cell stereocilia is defective in the mouse mutant whirler. *J Comp Neurol* 450:94-102.
- Hope CI, Bunday S, Proops D, Fielder AR (1997) Usher syndrome in the city of Birmingham-prevalence and clinical classification. *Br J Ophthalmol* 81:46-53.
- Joensuu T, Hamalainen R, Yuan B, Johnson C, Tegelberg S, Gasparini P, Zelante L, Pirvola U, Pakarinen L, Lehesjoki AE, de la Chapelle A, Sankila EM (2001) Mutations in a novel gene with transmembrane domains underlie Usher syndrome type 3. *Am J Hum Genet* 69:673-684.
- Johnson KR, Zheng QY, Weston MD, Ptacek LJ, Noben-Trauth K (2005) The Mass1frings mutation underlies early onset hearing impairment in BUB/BnJ mice, a model for the auditory pathology of Usher syndrome IIC. *Genomics* 85:582-590.
- Johnson KR, Gagnon LH, Webb LS, Peters LL, Hawes NL, Chang B, Zheng QY (2003) Mouse models of USH1C and DFNB18: phenotypic and molecular analyses of two new spontaneous mutations of the Ush1c gene. *Hum Mol Genet* 12:3075-3086.
- Kaiserman N, Obolensky A, Banin E, Sharon D (2007) Novel USH2A mutations in Israeli patients with retinitis pigmentosa and Usher syndrome type 2. *Arch Ophthalmol* 125:219-224.
- Kalay E, de Brouwer AP, Caylan R, Nabuurs SB, Wollnik B, Karaguzel A, Heister JG, Erdol H, Cremers FP, Cremers CW, Brunner HG, Kremer H (2005) A novel D458V mutation in the SANS PDZ binding motif causes atypical Usher syndrome. *J Mol Med (Berl)* 83:1025-1032.
- Kazmierczak P, Sakaguchi H, Tokita J, Wilson-Kubalek EM, Milligan RA, Muller U, Kachar B (2007) Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells. *Nature* 449:87-91.
- Keats BJ, Corey DP (1999) The usher syndromes. *Am J Med Genet* 89:158-166.

- Kikkawa Y, Mburu P, Morse S, Kominami R, Townsend S, Brown SD (2005) Mutant analysis reveals whirlin as a dynamic organizer in the growing hair cell stereocilium. *Hum Mol Genet* 14:391-400.
- Kikkawa Y, Shitara H, Wakana S, Kohara Y, Takada T, Okamoto M, Taya C, Kamiya K, Yoshikawa Y, Tokano H, Kitamura K, Shimizu K, Wakabayashi Y, Shiroishi T, Kominami R, Yonekawa H (2003) Mutations in a new scaffold protein Sans cause deafness in Jackson shaker mice. *Hum Mol Genet* 12:453-461.
- Kobayashi I, Imamura K, Kubota M, Ishikawa S, Yamada M, Tonoki H, Okano M, Storch WB, Moriuchi T, Sakiyama Y, Kobayashi K (1999) Identification of an autoimmune enteropathy-related 75-kilodalton antigen. *Gastroenterology* 117:823-830.
- Kremer H, van Wijk E, Marker T, Wolfrum U, Roepman R (2006) Usher syndrome: molecular links of pathogenesis, proteins and pathways. *Hum Mol Genet* 15 Spec No 2:R262-270.
- Kros CJ, Marcotti W, van Netten SM, Self TJ, Libby RT, Brown SD, Richardson GP, Steel KP (2002) Reduced climbing and increased slipping adaptation in cochlear hair cells of mice with Myo7a mutations. *Nat Neurosci* 5:41-47.
- Kussel-Andermann P, El-Amraoui A, Safieddine S, Nouaille S, Perfettini I, Lecuit M, Cossart P, Wolfrum U, Petit C (2000) Vezatin, a novel transmembrane protein, bridges myosin VIIA to the cadherin-catenins complex. *EMBO J* 19:6020-6029.
- Lagziel A, Ahmed ZM, Schultz JM, Morell RJ, Belyantseva IA, Friedman TB (2005) Spatiotemporal pattern and isoforms of cadherin 23 in wild type and waltzer mice during inner ear hair cell development. *Dev Biol* 280:295-306.
- Lagziel A, Overlack N, Bernstein SL, Morell RJ, Wolfrum U, Friedman TB (2009) Expression of cadherin 23 isoforms is not conserved: implications for a mouse model of Usher syndrome type 1D. *Mol Vis* 15:1843-1857.
- Lane PW (1963) Whirler Mice: A Recessive Behavior Mutation in Linkage Group Viii. *J Hered* 54:263-266.
- LaVail MM (1976) Rod outer segment disk shedding in rat retina: relationship to cyclic lighting. *Science* 194:1071-1074.
- Lefevre G, Michel V, Weil D, Lepelletier L, Bizard E, Wolfrum U, Hardelin JP, Petit C (2008) A core cochlear phenotype in USH1 mouse mutants implicates fibrous links of the hair bundle in its cohesion, orientation and differential growth. *Development* 135:1427-1437.
- Leibovici M, Safieddine S, Petit C (2008) Mouse models for human hereditary deafness. *Curr Top Dev Biol* 84:385-429.
- Lentz J, Pan F, Ng SS, Deininger P, Keats B (2007) Ush1c216A knock-in mouse survives Katrina. *Mutat Res* 616:139-144.
- Lentz JJ, Gordon WC, Farris HE, MacDonald GH, Cunningham DE, Robbins CA, Tempel BL, Bazan NG, Rubel EW, Oesterle EC, Keats BJ (2010) Deafness and retinal degeneration in a novel USH1C knock-in mouse model. *Dev Neurobiol* 70:253-267.
- Libby RT, Steel KP (2001) Electroretinographic anomalies in mice with mutations in Myo7a, the gene involved in human Usher syndrome type 1B. *Invest Ophthalmol Vis Sci* 42:770-778.
- Libby RT, Kitamoto J, Holme RH, Williams DS, Steel KP (2003) Cdh23 mutations in the mouse are associated with retinal dysfunction but not retinal degeneration. *Exp Eye Res* 77:731-739.

- Lillo C, Kitamoto J, Liu X, Quint E, Steel KP, Williams DS (2003) Mouse models for Usher syndrome 1B. *Adv Exp Med Biol* 533:143-150.
- Liu X, Udovichenko IP, Brown SD, Steel KP, Williams DS (1999) Myosin VIIa participates in opsin transport through the photoreceptor cilium. *J Neurosci* 19:6267-6274.
- Liu X, Bulgakov OV, Darrow KN, Pawlyk B, Adamian M, Liberman MC, Li T (2007) Usherin is required for maintenance of retinal photoreceptors and normal development of cochlear hair cells. *Proc Natl Acad Sci U S A* 104:4413-4418.
- Liu XZ, Walsh J, Mburu P, Kendrick-Jones J, Cope MJ, Steel KP, Brown SD (1997) Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nat Genet* 16:188-190.
- Liu XZ, Angeli SI, Rajput K, Yan D, Hodges AV, Eshraghi A, Telischi FF, Balkany TJ (2008) Cochlear implantation in individuals with Usher type 1 syndrome. *International Journal of Pediatric Otorhinolaryngology* 72:841-847.
- Lopes VS, Gibbs D, Libby RT, Aleman TS, Welch DL, Lillo C, Jacobson SG, Radu RA, Steel KP, Williams DS (2011) The Usher 1B protein, MYO7A, is required for normal localization and function of the visual retinoid cycle enzyme, RPE65. *Hum Mol Genet* 20:2560-2570.
- Lu B, Wang S, Francis PJ, Li T, Gamm DM, Capowski EE, Lund RD (2009) Cell transplantation to arrest early changes in an *ush2a* animal model. *Invest Ophthalmol Vis Sci* 51:2269-2276.
- Maerker T, van Wijk E, Overlack N, Kersten FF, McGee J, Goldmann T, Sehn E, Roepman R, Walsh EJ, Kremer H, Wolfrum U (2008) A novel Usher protein network at the periciliary reloading point between molecular transport machineries in vertebrate photoreceptor cells. *Hum Mol Genet* 17:71-86.
- Malm E, Ponjavic V, Moller C, Kimberling WJ, Andreasson S (2010) Phenotypes in Defined Genotypes Including Siblings with Usher Syndrome. *Ophthalmic Genet*.
- Malm E, Ponjavic V, Moller C, Kimberling WJ, Stone ES, Andreasson S (2011) Alteration of rod and cone function in children with Usher syndrome. *Eur J Ophthalmol* 21:30-38.
- Manji SS, Miller KA, Williams LH, Andreasen L, Siboe M, Rose E, Bahlo M, Kuiper M, Dahl HH (2011) An ENU-Induced Mutation of *Cdh23* Causes Congenital Hearing Loss, but No Vestibular Dysfunction, in Mice. *Am J Pathol* 179:903-914.
- Mazelova J, Ransom N, Astuto-Gribble L, Wilson MC, Deretic D (2009) Syntaxin 3 and SNAP-25 pairing, regulated by omega-3 docosahexaenoic acid, controls the delivery of rhodopsin for the biogenesis of cilia-derived sensory organelles, the rod outer segments. *J Cell Sci* 122:2003-2013.
- Mburu P, Liu XZ, Walsh J, Saw D, Jr., Cope MJ, Gibson F, Kendrick-Jones J, Steel KP, Brown SD (1997) Mutation analysis of the mouse myosin VIIA deafness gene. *Genes Funct* 1:191-203.
- Mburu P et al. (2003) Defects in whirlin, a PDZ domain molecule involved in stereocilia elongation, cause deafness in the whirler mouse and families with DFNB31. *Nat Genet* 34:421-428.
- McGee J, Goodyear RJ, McMillan DR, Stauffer EA, Holt JR, Locke KG, Birch DG, Legan PK, White PC, Walsh EJ, Richardson GP (2006) The very large G-protein-coupled receptor VLGR1: a component of the ankle link complex required for the normal development of auditory hair bundles. *J Neurosci* 26:6543-6553.

- McGee TL, Seyedahmadi BJ, Sweeney MO, Dryja TP, Berson EL (2010) Novel mutations in the long isoform of the USH2A gene in patients with Usher syndrome type II or non-syndromic retinitis pigmentosa. *J Med Genet* 47:499-506.
- McMillan DR, White PC (2004) Loss of the transmembrane and cytoplasmic domains of the very large G-protein-coupled receptor-1 (VLGR1 or Mass1) causes audiogenic seizures in mice. *Mol Cell Neurosci* 26:322-329.
- McMillan DR, White PC (2011) Studies on the very large g protein-coupled receptor: from initial discovery to determining its role in sensorineural deafness in higher animals. *Adv Exp Med Biol* 706:76-86.
- McMillan DR, Kayes-Wandover KM, Richardson JA, White PC (2002) Very large G protein-coupled receptor-1, the largest known cell surface protein, is highly expressed in the developing central nervous system. *J Biol Chem* 277:785-792.
- Michalski N, Michel V, Bahloul A, Lefevre G, Barral J, Yagi H, Chardenoux S, Weil D, Martin P, Hardelin JP, Sato M, Petit C (2007) Molecular characterization of the ankle-link complex in cochlear hair cells and its role in the hair bundle functioning. *J Neurosci* 27:6478-6488.
- Michalski N, Michel V, Caberlotto E, Lefevre GM, van Aken AF, Tinevez JY, Bizard E, Houbbron C, Weil D, Hardelin JP, Richardson GP, Kros CJ, Martin P, Petit C (2009) Harmonin-b, an actin-binding scaffold protein, is involved in the adaptation of mechanoelectrical transduction by sensory hair cells. *Pflugers Arch* 459:115-130.
- Michel V, Goodyear RJ, Weil D, Marcotti W, Perfettini I, Wolfrum U, Kros CJ, Richardson GP, Petit C (2005) Cadherin 23 is a component of the transient lateral links in the developing hair bundles of cochlear sensory cells. *Dev Biol* 280:281-294.
- Millan JM, Aller E, Jaijo T, Blanco-Kelly F, Gimenez-Pardo A, Ayuso C (2011) An update on the genetics of usher syndrome. *J Ophthalmol* 2011:417217.
- Nakanishi H, Ohtsubo M, Iwasaki S, Hotta Y, Mizuta K, Mineta H, Minoshima S (2009) Identification of 11 novel mutations in USH2A among Japanese patients with Usher syndrome type 2. *Clin Genet* 76:383-391.
- Nayak GD, Ratnayaka HS, Goodyear RJ, Richardson GP (2007) Development of the hair bundle and mechanotransduction. *Int J Dev Biol* 51:597-608.
- Nicolson T, Rusch A, Friedrich RW, Granato M, Ruppertsberg JP, Nusslein-Volhard C (1998) Genetic analysis of vertebrate sensory hair cell mechanosensation: the zebrafish circler mutants. *Neuron* 20:271-283.
- Nikkila H, McMillan DR, Nunez BS, Pascoe L, Curnow KM, White PC (2000) Sequence similarities between a novel putative G protein-coupled receptor and Na⁺/Ca²⁺ exchangers define a cation binding domain. *Mol Endocrinol* 14:1351-1364.
- Noben-Trauth K, Zheng QY, Johnson KR (2003) Association of cadherin 23 with polygenic inheritance and genetic modification of sensorineural hearing loss. *Nat Genet* 35:21-23.
- Nudelman I, Glikin D, Smolkin B, Hainrichson M, Belakhov V, Baasov T (2010) Repairing faulty genes by aminoglycosides: development of new derivatives of geneticin (G418) with enhanced suppression of diseases-causing nonsense mutations. *Bioorg Med Chem* 18:3735-3746.
- Nudelman I, Rebibo-Sabbah A, Cherniavsky M, Belakhov V, Hainrichson M, Chen F, Schacht J, Pilch DS, Ben-Yosef T, Baasov T (2009) Development of novel

- aminoglycoside (NB54) with reduced toxicity and enhanced suppression of disease-causing premature stop mutations. *J Med Chem* 52:2836-2845.
- Osten P, Stern-Bach Y (2006) Learning from stargazin: the mouse, the phenotype and the unexpected. *Curr Opin Neurobiol* 16:275-280.
- Pakarinen L, Tuppurainen K, Laippala P, Mäntylä M, Puhakka H (1995) The ophthalmological course of Usher syndrome type III. *Int Ophthalmol* 19:307-311.
- Pan L, Yan J, Wu L, Zhang M (2009) Assembling stable hair cell tip link complex via multidentate interactions between harmonin and cadherin 23. *Proc Natl Acad Sci U S A* 106:5575-5580.
- Papermaster DS (2002) The birth and death of photoreceptors: the Friedenwald Lecture. *Invest Ophthalmol Vis Sci* 43:1300-1309.
- Papermaster DS, Schneider BG, DeFoe D, Besharse JC (1986) Biosynthesis and vectorial transport of opsin on vesicles in retinal rod photoreceptors. *J Histochem Cytochem* 34:5-16.
- Pearsall N, Bhattacharya G, Wisecarver J, Adams J, Cosgrove D, Kimberling W (2002) Usherin expression is highly conserved in mouse and human tissues. *Hear Res* 174:55-63.
- Peng YW, Zallocchi M, Wang WM, Delimont D, Cosgrove D (2011) Moderate light induced degeneration of rod photoreceptors with delayed transducin translocation in shaker1 mice. *Invest Ophthalmol Vis Sci*.
- Pennings RJ, Damen GW, Snik AF, Hoefsloot L, Cremers CW, Mylanus EA (2006) Audiologic performance and benefit of cochlear implantation in Usher syndrome type I. *Laryngoscope* 116:717-722.
- Peters KR, Palade GE, Schneider BG, Papermaster DS (1983) Fine structure of a periciliary ridge complex of frog retinal rod cells revealed by ultrahigh resolution scanning electron microscopy. *J Cell Biol* 96:265-276.
- Petit C (2001) Usher syndrome: from genetics to pathogenesis. *Annu Rev Genomics Hum Genet* 2:271-297.
- Phillips JB, Blanco-Sanchez B, Lentz JJ, Tallafuss A, Khanobdee K, Sampath S, Jacobs ZG, Han PF, Mishra M, Williams DS, Keats BJ, Washbourne P, Westerfield M (2011) Harmonin (Ush1c) is required in zebrafish Muller glial cells for photoreceptor synaptic development and function. *Dis Model Mech*.
- Rana MW, Taraszka SR (1991) Monkey photoreceptor calycal processes and interphotoreceptor matrix as observed by scanning electron microscopy. *Am J Anat* 192:472-477.
- Rebibo-Sabbah A, Nudelman I, Ahmed ZM, Baasov T, Ben-Yosef T (2007) In vitro and ex vivo suppression by aminoglycosides of PCDH15 nonsense mutations underlying type 1 Usher syndrome. *Hum Genet* 122:373-381.
- Reiners J, Marker T, Jurgens K, Reidel B, Wolfrum U (2005a) Photoreceptor expression of the Usher syndrome type 1 protein protocadherin 15 (USH1F) and its interaction with the scaffold protein harmonin (USH1C). *Mol Vis* 11:347-355.
- Reiners J, Nagel-Wolfrum K, Jurgens K, Marker T, Wolfrum U (2006) Molecular basis of human Usher syndrome: deciphering the meshes of the Usher protein network provides insights into the pathomechanisms of the Usher disease. *Exp Eye Res* 83:97-119.

- Reiners J, Reidel B, El-Amraoui A, Boeda B, Huber I, Petit C, Wolfrum U (2003) Differential distribution of harmonin isoforms and their possible role in Usher-1 protein complexes in mammalian photoreceptor cells. *Invest Ophthalmol Vis Sci* 44:5006-5015.
- Reiners J, van Wijk E, Marker T, Zimmermann U, Jurgens K, te Brinke H, Overlack N, Roepman R, Knipper M, Kremer H, Wolfrum U (2005b) Scaffold protein harmonin (USH1C) provides molecular links between Usher syndrome type 1 and type 2. *Hum Mol Genet* 14:3933-3943.
- Rhodes CR, Hertzano R, Fuchs H, Bell RE, de Angelis MH, Steel KP, Avraham KB (2004) A Myo7a mutation cosegregates with stereocilia defects and low-frequency hearing impairment. *Mamm Genome* 15:686-697.
- Richardson GP, de Monvel JB, Petit C (2011) How the genetics of deafness illuminates auditory physiology. *Annu Rev Physiol* 73:311-334.
- Rivolta C, Berson EL, Dryja TP (2002) Paternal uniparental heterodisomy with partial isodisomy of chromosome 1 in a patient with retinitis pigmentosa without hearing loss and a missense mutation in the Usher syndrome type II gene USH2A. *Arch Ophthalmol* 120:1566-1571.
- Rodriguez de Turco EB, Deretic D, Bazan NG, Papermaster DS (1997) Post-Golgi vesicles cotransport docosahexaenoyl-phospholipids and rhodopsin during frog photoreceptor membrane biogenesis. *J Biol Chem* 272:10491-10497.
- Roepman R, Wolfrum U (2007) Protein networks and complexes in photoreceptor cilia. *Subcell Biochem* 43:209-235.
- Rosenberg T, Haim M, Hauch AM, Parving A (1997) The prevalence of Usher syndrome and other retinal dystrophy-hearing impairment associations. *Clin Genet* 51:314-321.
- Rzadzinska AK, Derr A, Kachar B, Noben-Trauth K (2005) Sustained cadherin 23 expression in young and adult cochlea of normal and hearing-impaired mice. *Hear Res* 208:114-121.
- Sadeghi AM, Eriksson K, Kimberling WJ, Sjoström A, Møller C (2006) Longterm visual prognosis in Usher syndrome types 1 and 2. *Acta Ophthalmol Scand* 84:537-544.
- Saihan Z, Webster AR, Luxon L, Bitner-Glindzicz M (2009) Update on Usher syndrome. *Curr Opin Neurol* 22:19-27.
- Sandberg MA, Rosner B, Weigel-DiFranco C, McGee TL, Dryja TP, Berson EL (2008) Disease course in patients with autosomal recessive retinitis pigmentosa due to the USH2A gene. *Invest Ophthalmol Vis Sci* 49:5532-5539.
- Scanlan MJ, Williamson B, Jungbluth A, Stockert E, Arden KC, Viars CS, Gure AO, Gordan JD, Chen YT, Old LJ (1999) Isoforms of the human PDZ-73 protein exhibit differential tissue expression. *Biochim Biophys Acta* 1445:39-52.
- Schneider E, Marker T, Daser A, Frey-Mahn G, Beyer V, Farcas R, Schneider-Ratzke B, Kohlschmidt N, Grossmann B, Bauss K, Napióntek U, Keilmann A, Bartsch O, Zechner U, Wolfrum U, Haaf T (2009) Homozygous disruption of PDZD7 by reciprocal translocation in a consanguineous family: a new member of the Usher syndrome protein interactome causing congenital hearing impairment. *Hum Mol Genet* 18:655-666.
- Schwander M, Xiong W, Tokita J, Lelli A, Elledge HM, Kazmierczak P, Sczaniecka A, Kolatkar A, Wiltshire T, Kuhn P, Holt JR, Kachar B, Tarantino L, Müller U (2009) A

- mouse model for nonsyndromic deafness (DFNB12) links hearing loss to defects in tip links of mechanosensory hair cells. *Proc Natl Acad Sci U S A* 106:5252-5257.
- Seiler C, Finger-Baier KC, Rinner O, Makhankov YV, Schwarz H, Neuhauss SC, Nicolson T (2005) Duplicated genes with split functions: independent roles of protocadherin15 orthologues in zebrafish hearing and vision. *Development* 132:615-623.
- Self T, Mahony M, Fleming J, Walsh J, Brown SD, Steel KP (1998) Shaker-1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells. *Development* 125:557-566.
- Senften M, Schwander M, Kazmierczak P, Lillo C, Shin JB, Hasson T, Geleoc GS, Gillespie PG, Williams D, Holt JR, Muller U (2006) Physical and functional interaction between protocadherin 15 and myosin VIIa in mechanosensory hair cells. *J Neurosci* 26:2060-2071.
- Seyedahmadi BJ, Rivolta C, Keene JA, Berson EL, Dryja TP (2004) Comprehensive screening of the USH2A gene in Usher syndrome type II and non-syndromic recessive retinitis pigmentosa. *Exp Eye Res* 79:167-173.
- Sheng M, Sala C (2001) PDZ domains and the organization of supramolecular complexes. *Annu Rev Neurosci* 24:1-29.
- Siemens J, Kazmierczak P, Reynolds A, Sticker M, Littlewood-Evans A, Muller U (2002) The Usher syndrome proteins cadherin 23 and harmonin form a complex by means of PDZ-domain interactions. *Proc Natl Acad Sci U S A* 99:14946-14951.
- Siemens J, Lillo C, Dumont RA, Reynolds A, Williams DS, Gillespie PG, Muller U (2004) Cadherin 23 is a component of the tip link in hair-cell stereocilia. *Nature* 428:950-955.
- Skradski SL, Clark AM, Jiang H, White HS, Fu YH, Ptacek LJ (2001) A novel gene causing a mendelian audiogenic mouse epilepsy. *Neuron* 31:537-544.
- Slepek VZ, Hurley JB (2008) Mechanism of light-induced translocation of arrestin and transducin in photoreceptors: interaction-restricted diffusion. *IUBMB Life* 60:2-9.
- Smith RJ, Berlin CI, Hejtmancik JF, Keats BJ, Kimberling WJ, Lewis RA, Moller CG, Pelias MZ, Tranebjaerg L (1994) Clinical diagnosis of the Usher syndromes. Usher Syndrome Consortium. *Am J Med Genet* 50:32-38.
- Smits BM, Peters TA, Mul JD, Croes HJ, Fransen JA, Beynon AJ, Guryev V, Plasterk RH, Cuppen E (2005) Identification of a rat model for usher syndrome type 1B by N-ethyl-N-nitrosourea mutagenesis-driven forward genetics. *Genetics* 170:1887-1896.
- Sollner C, Rauch GJ, Siemens J, Geisler R, Schuster SC, Muller U, Nicolson T (2004) Mutations in cadherin 23 affect tip links in zebrafish sensory hair cells. *Nature* 428:955-959.
- Spandau UH, Rohrschneider K (2002) Prevalence and geographical distribution of Usher syndrome in Germany. *Graefes Arch Clin Exp Ophthalmol* 240:495-498.
- Tian C, Liu XZ, Han F, Yu H, Longo-Guess C, Yang B, Lu C, Yan D, Zheng QY (2010) Ush1c gene expression levels in the ear and eye suggest different roles for Ush1c in neurosensory organs in a new Ush1c knockout mouse. *Brain Res* 1328:57-70.
- Tian G, Zhou Y, Hajkova D, Miyagi M, Dinculescu A, Hauswirth WW, Palczewski K, Geng R, Alagramam KN, Isosomppi J, Sankila EM, Flannery JG, Imanishi Y (2009) Clarin-1, encoded by the Usher Syndrome III causative gene, forms a membranous microdomain: possible role of clarin-1 in organizing the actin cytoskeleton. *J Biol Chem* 284:18980-18993.

- Tlili A, Charfedine I, Lahmar I, Benzina Z, Mohamed BA, Weil D, Idriss N, Drira M, Masmoudi S, Ayadi H (2005) Identification of a novel frameshift mutation in the DFNB31/WHRN gene in a Tunisian consanguineous family with hereditary non-syndromic recessive hearing loss. *Hum Mutat* 25:503.
- Tomita S, Shenoy A, Fukata Y, Nicoll RA, Brecht DS (2007) Stargazin interacts functionally with the AMPA receptor glutamate-binding module. *Neuropharmacology* 52:87-91.
- Usher CH (1914) On the inheritance of retinitis pigmentosa, with notes of cases. *R Lond Ophthalmol Hosp Rep* 19:130-236.
- van Wijk E, Pennings RJ, te Brinke H, Claassen A, Yntema HG, Hoefsloot LH, Cremers FP, Cremers CW, Kremer H (2004) Identification of 51 novel exons of the Usher syndrome type 2A (USH2A) gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. *Am J Hum Genet* 74:738-744.
- van Wijk E, van der Zwaag B, Peters T, Zimmermann U, Te Brinke H, Kersten FF, Marker T, Aller E, Hoefsloot LH, Cremers CW, Cremers FP, Wolfrum U, Knipper M, Roepman R, Kremer H (2006) The DFNB31 gene product whirlin connects to the Usher protein network in the cochlea and retina by direct association with USH2A and VLGR1. *Hum Mol Genet* 15:751-765.
- Vastinsalo H, Jalkanen R, Dinculescu A, Isosomppi J, Geller S, Flannery JG, Hauswirth WW, Sankila EM (2010) Alternative splice variants of the USH3A gene Clarin 1 (CLRN1). *Eur J Hum Genet* 19:30-35.
- Verpy E, Leibovici M, Zwaenepoel I, Liu XZ, Gal A, Salem N, Mansour A, Blanchard S, Kobayashi I, Keats BJ, Slim R, Petit C (2000) A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C. *Nat Genet* 26:51-55.
- Vollrath MA, Kwan KY, Corey DP (2007) The micromachinery of mechanotransduction in hair cells. *Annu Rev Neurosci* 30:339-365.
- von Graefe A (1858) Vereinzelte Beobachtungen und Bemerkungen. Exceptionelles Verhalten des Gesichtsfeldes bei Pigmententartung des Netzhaut. *Albrecht Graefes Arch Klin Ophthalmol* 4:250-253.
- Wada T, Wakabayashi Y, Takahashi S, Ushiki T, Kikkawa Y, Yonekawa H, Kominami R (2001) A point mutation in a cadherin gene, Cdh23, causes deafness in a novel mutant, Waltzer mouse niigata. *Biochem Biophys Res Commun* 283:113-117.
- Washington JL, 3rd, Pitts D, Wright CG, Erway LC, Davis RR, Alagramam K (2005) Characterization of a new allele of Ames waltzer generated by ENU mutagenesis. *Hear Res* 202:161-169.
- Webb SW, Grillet N, Andrade LR, Xiong W, Swarthout L, Della Santina CC, Kachar B, (2011) Muller U Regulation of PCDH15 function in mechanosensory hair cells by alternative splicing of the cytoplasmic domain. *Development* 138:1607-1617.
- Weil D, Kussel P, Blanchard S, Levy G, Levi-Acobas F, Drira M, Ayadi H, Petit C (1997) The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nat Genet* 16:191-193.
- Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, Mburu P, Varela A, Levilliers J, Weston MD, et al. (1995) Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* 374:60-61.

- Weil D, El-Amraoui A, Masmoudi S, Mustapha M, Kikkawa Y, Laine S, Delmaghani S, Adato A, Nadifi S, Zina ZB, Hamel C, Gal A, Ayadi H, Yonekawa H, Petit C (2003) Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. *Hum Mol Genet* 12:463-471.
- Weston MD, Luijendijk MW, Humphrey KD, Moller C, Kimberling WJ (2004) Mutations in the VLGR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. *Am J Hum Genet* 74:357-366.
- Weston MD, Eudy JD, Fujita S, Yao S, Usami S, Cremers C, Greenberg J, Ramesar R, Martini A, Moller C, Smith RJ, Sumegi J, Kimberling WJ (2000) Genomic structure and identification of novel mutations in usherin, the gene responsible for Usher syndrome type IIa. *Am J Hum Genet* 66:1199-1210.
- Williams DS (2008) Usher syndrome: animal models, retinal function of Usher proteins, and prospects for gene therapy. *Vision Res* 48:433-441.
- Williams DS, Aleman TS, Lillo C, Lopes VS, Hughes LC, Stone EM, Jacobson SG (2009) Harmonin in the murine retina and the retinal phenotypes of Ush1c-mutant mice and human USH1C. *Invest Ophthalmol Vis Sci* 50:3881-3889.
- Wilson SM, Householder DB, Coppola V, Tessarollo L, Fritzsche B, Lee EC, Goss D, Carlson GA, Copeland NG, Jenkins NA (2001) Mutations in Cdh23 cause nonsyndromic hearing loss in waltzer mice. *Genomics* 74:228-233.
- Wolfrum U, Liu X, Schmitt A, Udovichenko IP, Williams DS (1998) Myosin VIIa as a common component of cilia and microvilli. *Cell Motil Cytoskeleton* 40:261-271.
- Wu L, Pan L, Wei Z, Zhang M (2011) Structure of MyTH4-FERM domains in myosin VIIa tail bound to cargo. *Science* 331:757-760.
- Yagi H, Takamura Y, Yoneda T, Konno D, Akagi Y, Yoshida K, Sato M (2005) Vlgr1 knockout mice show audiogenic seizure susceptibility. *J Neurochem* 92:191-202.
- Yagi H, Tokano H, Maeda M, Takabayashi T, Nagano T, Kiyama H, Fujieda S, Kitamura K, Sato M (2007) Vlgr1 is required for proper stereocilia maturation of cochlear hair cells. *Genes Cells* 12:235-250.
- Yan D, Kamiya K, Ouyang XM, Liu XZ (2011) Analysis of subcellular localization of Myo7a, Pcdh15 and Sans in Ush1c knockout mice. *Int J Exp Pathol* 92:66-71.
- Yan D, Ouyang X, Patterson DM, Du LL, Jacobson SG, Liu XZ (2009) Mutation analysis in the long isoform of USH2A in American patients with Usher Syndrome type II. *J Hum Genet* 54:732-738.
- Yan J, Pan L, Chen X, Wu L, Zhang M (2010) The structure of the harmonin/sans complex reveals an unexpected interaction mode of the two Usher syndrome proteins. *Proc Natl Acad Sci U S A* 107:4040-4045.
- Yang J, Wang L, Song H, Sokolov M (2011) Current understanding of usher syndrome type II. *Frontiers in Bioscience* 17:1165-1183.
- Yang J, Liu X, Zhao Y, Adamian M, Pawlyk B, Sun X, McMillan DR, Liberman MC, Li T (2010) Ablation of whirlin long isoform disrupts the USH2 protein complex and causes vision and hearing loss. *PLoS Genet* 6:e1000955.
- Yonezawa S, Yoshizaki N, Kageyama T, Takahashi T, Sano M, Tokita Y, Masaki S, Inaguma Y, Hanai A, Sakurai N, Yoshiki A, Kusakabe M, Moriyama A, Nakayama A (2006) Fates of Cdh23/CDH23 with mutations affecting the cytoplasmic region. *Hum Mutat* 27:88-97.

- Young RW (1967) The renewal of photoreceptor cell outer segments. *J Cell Biol* 33:61-72.
- Young RW (1976) Visual cells and the concept of renewal. *Invest Ophthalmol Vis Sci* 15:700-725.
- Zallocchi M, Meehan DT, Delimont D, Askew C, Garige S, Gratton MA, Rothermund-Franklin CA, Cosgrove D (2009) Localization and expression of clarin-1, the *Clrn1* gene product, in auditory hair cells and photoreceptors. *Hear Res* 255:109-120.
- Zou J, Luo L, Shen Z, Chiodo VA, Ambati BK, Hauswirth WW, Yang J (2011) Whirlin Replacement Restores the Formation of the USH2 Protein Complex in Whirlin Knockout Photoreceptors. *Invest Ophthalmol Vis Sci* 52:2343-2351.



Hearing Loss

Edited by Dr. Sadaf Naz

ISBN 978-953-51-0366-0

Hard cover, 406 pages

Publisher InTech

Published online 28, March, 2012

Published in print edition March, 2012

Authored by 17 international researchers and research teams, the book provides up-to-date insights on topics in five different research areas related to normal hearing and deafness. Techniques for assessment of hearing and the appropriateness of the Mongolian gerbil as a model for age-dependent hearing loss in humans are presented. Parental attitudes to childhood deafness and role of early intervention for better treatment of hearing loss are also discussed. Comprehensive details are provided on the role of different environmental insults including injuries in causing deafness. Additionally, many genes involved in hearing loss are reviewed and the genetics of recessively inherited moderate to severe and progressive deafness is covered for the first time. The book also details established and evolving therapies for treatment of deafness.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jun Yang (2012). Usher Syndrome: Genes, Proteins, Models, Molecular Mechanisms, and Therapies, Hearing Loss, Dr. Sadaf Naz (Ed.), ISBN: 978-953-51-0366-0, InTech, Available from:

<http://www.intechopen.com/books/hearing-loss/usher-syndrome-genes-proteins-models-molecular-mechanisms-and-therapies>

INTeCH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen