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Function and expression pattern of nonsyndromic deafness genes

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Abstract

Hearing loss is the most common sensory disorder, present in 1 of every 500 newborns. To date, 46 genes have been identified that cause nonsyndromic hearing loss, making it an extremely heterogeneous trait. This review provides a comprehensive overview of the inner ear function and expression pattern of these genes. In general, they are involved in hair bundle morphogenesis, form constituents of the extracellular matrix, play a role in cochlear ion homeostasis or serve as transcription factors. During the past few years, our knowledge of genes involved in hair bundle morphogenesis has increased substantially. We give an up-to-date overview of both the nonsyndromic and Usher syndrome genes involved in this process, highlighting proteins that interact to form macromolecular complexes. For every gene, we also summarize its expression pattern and impact on hearing at the functional level. Gene-specific cochlear expression is summarized in a unique table by structure/cell type and is illustrated on a cochlear cross-section, which is available online via the Hereditary Hearing Loss Homepage. This review should provide auditory scientists the most relevant information for all identified nonsyndromic deafness genes.

Keywords

Hereditary hearing loss; ARNSHL; ADNSHL; hair bundle; ion homeostasis; cochlear expression pattern

Introduction

Hearing loss (HL) is the most common birth defect and the most prevalent sensorineural disorder in developed countries. One of every 500 newborns is affected with bilateral congenital sensorineural hearing loss ≥ 40 dB HL. This number rises to 2.7 per 1000 before the age of 5 and 3.5 per 1000 during adolescence [1]. If the HL starts before speech development, it is called prelingual, with congenital HL indicating the presence of HL at birth. Postlingual HL starts after speech development. The most common example is age-related hearing loss, which affects about half of octogenarians.

HL can be conductive (outer or middle ear defect), sensorineural (inner ear defect) or mixed (a combination of both conductive and sensorineural defects). It is estimated that HL is caused by genetic factors in at least two-thirds of prelingual cases in developed countries. The remaining cases are attributed to environmental factors, such as congenital cytomegaloviral infection, or unrecognized genetic factors. If HL is inherited, it is almost always monogenic.

70% of inherited cases are nonsyndromic, with HL being the only disorder. Over 400 syndromic forms of HL have been described in which HL is associated with other abnormalities. The most common examples are Usher syndrome, Pendred syndrome and Jervell and Lange-Nielsen syndrome [2,3].

HL can also be multifactorial or complex in causality, reflecting the interaction of a number of genetic and environmental factors. Age-related hearing loss is the most frequent example of a genetically complex type of hearing loss. To date, only a few genes have been associated with these types of HL. It is unclear whether genes involved in monogenic forms of HL play a role in complex HL.

Monogenic hearing loss

The inheritance pattern of monogenic nonsyndromic HL is autosomal recessive (ARNSHL) in ~80% of cases, autosomal dominant (ADNSHL) in ~20% of cases, X-linked (<1% of cases) or mitochondrial (<<1% of cases). ARNSHL is typically prelingual, while ADNSHL is usually postlingual [4]. The genetic heterogeneity of HL is extremely high: 52 ARNSHL loci have been mapped and 28 deafness genes identified, 43 ADNSHL loci have been mapped and 22 genes have been identified, 4 X-linked nonsyndromic HL loci have been mapped with one gene identification and mutations in two mitochondrial genes also cause nonsyndromic HL (Hereditary Hearing Loss Homepage: <http://webh01.ua.ac.be/hhh/>). The majority of these gene identifications were accomplished by positional cloning, although the identification and characterization of new deafness genes in mouse mutants has permitted successful candidate gene screening in a number of cases.

Deafness genes and their function in the hearing process

Our knowledge of the proteome of the inner ear has been fueled considerably by the identification of genes responsible for hereditary deafness in humans. The discovery of genes biologically relevant to auditory function has been complemented by the analysis of spontaneous and induced mouse models of hereditary hearing loss, as well as the study of non-mammalian species. These genes fall into four broad functional categories: hair bundle morphogenesis, ion homeostasis, extracellular matrix composition and transcription factors. For eight deafness genes, a definite function has not been determined. Here we provide a complete overview of the function and expression pattern of all 46 nonsyndromic deafness genes, categorized according to their function in the hearing process, which is summarized in Table 1. Table 2 and Box 1 summarize their expression pattern.

Box 1

Expression pattern of nonsyndromic deafness genes available online at the Hereditary Hearing loss Homepage

The Hereditary Hearing loss Homepage (<http://webh01.ua.ac.be/hhh/>) is an important webpage, providing up-to-date information on the genetics of hearing loss. It provides an overview of all mapped loci and identified deafness genes, with references to the original research articles. All mapped loci and identified deafness genes are listed. Under the tab 'expression', 44 nonsyndromic deafness genes (excluding the two mitochondrial genes) are listed and linked to the figure below (Fig. (2) of this review), which shows the specific cochlear expression pattern for each gene. For genes expressed in the stereocilia of hair cells, an additional link is provided, indicating the exact location and interaction of the corresponding proteins (Fig. (1) of this review). For every gene, references are provided.

Table 2 of this review gives an overview of the expression pattern, categorized by structure/cell type and uses the same numbers as Fig. 2 to indicate the structures and cell types.

Hair bundle morphogenesis

Inner ear hair cells have a very specialized apical structure, the hair bundle (Fig. (1)). Each hair bundle is composed of stereocilia, which are microvilli-derived structures filled with cross-linked F-actin filaments. The stereocilia have a staircase-like pattern, with the kinocilium being the longest stereocilium, followed by rows of stereocilia of decreasing height. At the base of each stereocilium is the cuticular plate, which is an anchor of dense actin fibers together with other cytoskeletal proteins. The adherens junction connects hair cells to their neighboring supporting cells, and the pericuticular necklace lies between the adherens junction and the cuticular plate.

The stereocilia of the hair bundle are interconnected by four distinct types of links. From top to bottom there are tip links, horizontal top links, side links and ankle links. Only a single tip link connects the tips of two adjacent stereocilia. All other links are present in multiple copies and interconnect adjacent stereocilia within and across the rows. The horizontal top links are present just below the tips, the side links are distributed evenly over the hair bundle surface, and ankle links are thin filaments present at the beginning of the stereocilia taper. These links are believed to provide cohesiveness to the stereocilia bundle and may mediate signalling events during morphogenesis [5]. Only the top and tip links remain present in mature cells, while the other links are transient and only present during hair bundle development. In the presence of a kinocilium, kinocilial links attach the kinocilium to adjacent stereocilia in the tallest row.

The majority of proteins thus far discovered to be involved in the morphogenesis of the hair bundle are encoded by genes that cause nonsyndromic HL and/or Usher syndrome. They fall into several functional categories including proteins that form the cytoskeleton, adhesion proteins, motor proteins and scaffolding proteins. The adhesion proteins include two members of the cadherin superfamily (cadherin 23 and protocadherin 15), a tetraspan protein (TMHS) and two USH2 proteins (usherin and VLGR1b). These proteins are all transmembrane proteins that link to actin filaments through interactions with scaffolding and motor proteins. The three scaffolding proteins identified in the stereocilia are whirlin, harmonin b and SANS. The motor proteins include five unconventional myosins: IIIA, VI, VIIA, IX and XVA.

Results of many studies suggest that the Usher syndrome proteins form a stereociliary protein interactome that includes the above listed proteins as well as the USH2B candidate NBC3 and maybe also the USH3A protein clarin-1 [6,7]. During hair cell development, this protein network may coordinate differentiation of the stereocilia. Myosin VIIA may transfer harmonin b to the stereocilia where it stabilizes the actin filaments and connects them to the transmembrane proteins of the network (CDH23, PCDH15, VLGR1b, USH2A and possibly NBC3). In mature hair cells, the network may be involved in mechano-electrical transduction (MET) in the stereocilia and in ribbon synapse functioning (Fig. (1)).

Proteins of the cytoskeleton

The cytoskeleton of hair cells is a specialised structure that arises by a series of morphological transformations associated with actin assembly. Along the length of the stereocilia, unipolar actin filaments occur as hexagonally packed bundles which are crosslinked by fimbrin and espin. This actin core is thought to be renewed continuously through an actin treadmill mechanism, giving rise to a very stable and rigid structure. The rootlet filaments anchor stereocilia into the cuticular plate at the apical surface of the hair cell. Microtubules in the

kinocilium and beneath the cuticular plate connect these structures to the axial hair cell cytoskeleton. Adherens junctions, which also consist of actin fibers, may act to stabilize the cuticular plate. Six human deafness genes are believed to be part of the cytoskeleton: *ACTG1*, *DIAPH*, *ESPN*, *RDX*, *TRIOBP* and *CCDC50*.

ACTG1, encoding γ -actin, is the predominant actin isoform in intestinal epithelial cells and in auditory hair cells, more specifically in the cuticular plate, adherens junctions and stereocilia. *ACTG1* mutations have been shown to cause ADNSHL, starting in the high frequencies during the first three decades and progressing to profound hearing loss across all frequencies. There is a degree of phenotypic variation in age of onset and progression rate that may be due to modifying environmental and genetic factors or to the type of mutation. All mutations in *ACTG1* are predicted to have only a modest effect on the structure and function of γ -actin, which may explain the late onset of the hearing loss [8]. These mutations may interfere with bundling, gelation or polymerization of actin and in this way reduce stability. It is also possible that myosin movement could be affected [9].

Diaphanous 1, encoded by *DIAPH1*, is the human homologue of the corresponding *Drosophila* gene and belongs to the formin family of proteins. The protein is probably involved in actin polymerization, although its precise localization in the inner ear is unknown. It may accelerate actin nucleation through an interaction with the barbed end of actin filaments in stereocilia. A single mutation in *DIAPH* is known to cause low-frequency hearing loss that starts during the first decade of life and progresses rather quickly to profound deafness involving all frequencies [10].

ESPN codes for the espin protein, which is an actin-bundling protein present in the parallel actin bundle (PAB) of the stereocilia of cochlear and vestibular hair cells [11]. Espins are believed to play a crucial role in length regulation and integrity of stereocilia by mediating the transition from loose-to-dense packing of actin filaments. Espin has different isoforms which function preferentially in certain hair cell types or during discrete phases of stereociliary bundle formation. The jerker mouse is a recessive *Espn* mutant and exhibits head-jerking movements, rapid circling and complete deafness [12]. In humans, mutations in *ESPN* were first found to cause ARNSHL in 2004 [13]. Patients show congenital, profound hearing loss with vestibular areflexia but without eye symptoms. One year later, *ESPN* mutations were also found to cause ADNSHL without vestibular involvement. Studies of the dominant mutations in a transfection model suggest that they cause a defect in the elongation or organization of stereocilia, confirming the proposed function of the protein [14].

Radixin is part of the ezrin/radixin/moesin (ERM) family which consists of three closely related proteins that function as cross-linkers between plasma membranes and actin filaments [15]. Radixin is present along the length of the stereocilia, mainly at the lower half. By linking the actin-cytoskeleton to adhesion proteins, the protein participates in the formation of the membrane-associated cytoskeleton. Recently, the *RDX* gene has been found to cause ARNSHL in three Pakistani families [16].

The TRIO and filamentous actin binding protein encoded by *TRIOBP* colocalizes with F-actin along the length of the stereocilia and is thought to be involved in actin cytoskeletal organization [17]. The gene has different isoforms, of which mutations in the longest isoform are the cause of profound ARNSHL [17,18].

CCDC50 is a gene that encodes a tyrosine-phosphorylated effector of EGF-mediated signalling, also named Ymer. A mutation in the gene has recently been identified to cause ADNSHL in a Spanish family. The hearing loss starts early with a moderate loss in the low and mid frequencies, and progresses to profound HL across all frequencies [19]. Ymer is associated with microtubule-based structures in different cells of the organ of Corti (nerve

fibers of the spiral ganglion, OHCs, pillar cells, Deiter's cells and cells of the stria vascularis). Mutations in the gene probably cause destabilization of the cytoskeleton. Its precise role in the EGF-mediated signalling pathway is currently unknown.

Adhesion proteins

Cadherin 23 and protocadherin 15 are members of the cadherin superfamily of integral membrane proteins which are responsible for intercellular adhesion and for signalling. Both proteins contain PDZ-binding motifs through which they bind the PDF domain of a scaffolding protein for anchoring the actin cytoskeleton.

CDH23 encodes for cadherin 23 and is expressed in the sensory hair cells and in the Reissner's membrane. In the developing hair cells, the protein is a component of transient lateral links between neighbouring stereocilia and is believed to play an essential role in cohesion of stereocilia during hair bundle development. Cadherin 23 is also a component of the tip links and kinocilial link in the mature hair cell [20]. *Cdh23* is the disease-causing gene in the deaf-waltzer (*v*) mouse mutant [21] and has been implicated in age-related hearing loss (AHL) in murine inbred strains [22]. In humans, *CDH23* mutations cause both Usher syndrome type ID (USH1D) and ARNSHL (DFNB12). According to a suggested genotype-phenotype correlation which is not absolute, missense mutations or in-frame alterations cause ARNSHL, while truncating mutations cause USH1D [23].

Protocadherin 15 has four major isoform classes, which have specific spatiotemporal expression patterns in developing and mature hair cells. In the fetal cochlea, *Pcdh15* is found in supporting cells, outer sulcus cells and the spiral ganglion. *Pcdh15*-CD2 is expressed along the length of the stereocilia during hair bundle development and is believed to be involved in the formation of the transient lateral links. Due to their expression pattern, *Pcdh15*-CD3 and *Pcdh15*-CD1 are believed to be associated with the tip-link complex [20,24]. The scaffolding protein between protocadherin 15 and the actin filaments has not been identified yet. In Ames waltzer (*av*) mice, recessive *Pcdh15* mutations cause a deafness phenotype due to disorganization of stereocilia bundles and degeneration of inner ear neuroepithelia. Mutations in *PCDH15* cause both Usher syndrome type 1F and severe-to-profound ARNSHL [25]. A genotype-phenotype correlation has been proposed for *PCDH15* mutations in which hypomorphic alleles of the gene cause ARNSHL and more severe mutations cause Usher syndrome.

TMHS encodes for the tetraspan membrane protein of hair cell stereocilia and was recently discovered in the recessive mouse mutant hurry-scurry (*hscy*), in which recessive *Tmhs* mutations cause hearing loss and vestibular dysfunction [26]. In this mouse mutant, the protein is located at the apical membrane of the hair cell and in stereocilia, with a higher concentration towards the tip and the highest levels in developing hair bundles. This expression pattern may indicate a role for *Tmhs* in hair bundle morphogenesis. As *Tmhs* has a similar expression pattern compared to *Cdh23* and other members of the tetraspan protein family have been shown to associate with cadherins, *Tmhs* may interact with *Cdh23* and be part of the USH protein complex [27]. Mutations in the human *TMHS* are the cause of profound ARNSHL without vestibular dysfunction [28].

VLGR1b (*GPR98*) is expressed in the synaptic region and in stereocilia of hair cells. It is the largest cell surface receptor known and contains different protein domains including CalX- β domains for cell-cell adhesion or extracellular Ca^{2+} monitoring [29]. The large ectodomain contains several repeat regions and a pentraxin homology domain, which is homologous to the one in USH2A. *VLGR1b* may bind to USH2A through their extracellular domains. Through its C-terminal part, *VLGR1b* interacts with harmonin [7].

USH2A is expressed in the basement membrane of the cochlea, in stereocilia and in synaptic regions. The protein contains several protein domains including a transmembrane domain and a cytoplasmic tail which interacts with harmonin.

All three proteins causing Usher syndrome type II, usherin (USH2A), VLGR1b (USH2C) and whirlin (USH2D), and the putative transmembrane protein vezatin have been suggested to be part of the ankle-link molecular complex (ALC) in the hair cells. The complex connects growing stereocilia in developing hair cells and is believed to be scaffolded by whirlin and conveyed to the stereocilia by myosin VIIA [30].

Motor proteins

All motor proteins identified as deafness genes are expressed in the inner ear hair cells and belong to the myosin superfamily. They are subdivided into conventional and unconventional myosins. Myosins are actin-based molecular motors that regulate several processes, such as rearrangement of the actin cytoskeleton, regulation of tension of actin filaments and transport of organelles [31]. Conventional myosins form filaments and regulate contractility of actin filaments, while the function of unconventional myosins is more varied and includes crucial cellular roles such as vesicle trafficking and endocytosis. Five unconventional myosins (IIIA, VI, VIIA, IX and XVA) have been implicated in deafness, occasionally with vestibular dysfunction. Each myosin probably has a hair-cell-specific function, as gene-specific mutations cause distinguishable hair cell phenotypes.

Myosin VI, encoded by *MYO6*, is expressed most strongly in the cuticular plate and is involved in stereocilia formation. The protein is required to attach the apical plasma membrane to the base of stereocilia and/or anchor stereocilia rootlets. Its unique function as a backward-stepping actin-based motor that moves towards the minus end of actin filaments suggests that myosin VI may facilitate the removal of molecular components that are released by treadmilling at the taper of the stereocilium [5]. Mutations in the murine homologue *Myo6* lead to fusion of stereocilia at their base and underlie deafness in the Snell's waltzer (*sv*) mouse. In humans, nonsense mutations in *MYO6* cause congenital, profound ARSNHL while missense mutations cause ADNSHL with a milder phenotype and a later onset, secondary to a dominant-negative mechanism of action [32]. In one family, a dominant *MYO6* mutation leads to a combination of ADNSHL and hypertrophic cardiomyopathy [33].

MYO7A encodes for myosin VIIA, which is ubiquitously expressed in many epithelial tissues including the inner ear and retina. In the inner ear, myosin VIIA is found in the sensory hair cells, mainly in the stereocilia but also along the lateral membrane of the cell, in the cuticular plate and in the synaptic region. The motor domain allows interactions with actin filaments and makes this protein an actin-based molecular motor. The tail domain contains a coiled-coil domain for homodimer formation and a FERM domain, which may allow attachment to the plasma membrane. Vezatin, harmonin and SANS interact with myosin VIIA through binding with the tail domain. Two mouse mutants carrying *Myo7a* mutations have been described: the recessive mutant shaker-1 (*sh1*) and the dominant mutant Headbanger (*Hdb*) [34,35]. Although both mutants show typical circling and head-tossing behaviour and have hearing loss, their inner ear phenotypes differ. *Sh1* mutants show progressively disorganised stereocilia, grouped in clumps. In *Hdb* mice, OHC stereocilia form O instead of V shapes and many IHC stereocilia fuse and elongate, forming giant stereocilia [35]. Studies of *sh1* show that *Myo7a* is essential for differentiation and organization of hair cell stereocilia [6] and may determine the exact length of stereocilia [36]. In addition, myosin VIIA may also be involved in MET and in the function of ribbon synapses. In humans, mutations in *MYO7A* cause ADNSHL occasionally associated with vestibular disorders (DFNA11), profound ARNSHL (DFNB2), Usher syndrome type 1B (USH1B) and USH3-like phenotypes [37,38]. One missense mutation in

MYO7A also has been reported to cause low-frequency hearing loss, as is seen in the Headbanger mutant [39].

MYH9 encodes for the non-muscle myosin heavy-chain 9 protein and is expressed in fibrocytes of the spiral ligament and spiral limbus, along the length of the stereocilia, in the cuticular plate of sensory hair cells and minimally in the spiral ganglion [40]. However, its specific role in the cochlea is unknown. Based on its expression in fibrocytes, *Myh9* may be involved in anchoring surrounding cells and/or modulating/reacting to tension generated in the basilar membrane–spiral ligament complex. Its presence in the stereocilia suggests a role in maintenance of stereocilia structure or function [41]. No spontaneous mouse model exists for *Myh9* mutations, as mice carrying a gene-trap insertion in *Myh9* die *in utero*. Only one mutation in the human *MYH9* gene has been reported to cause progressive moderate-to-severe ADNSHL [42], although 27 different *MYH9* mutations have been described. The associated diseases are clinically heterogeneous, but include macrothrombocytopenia at birth in all patients. Other symptoms that can develop are sensorineural hearing loss, nephritis that progresses to end-stage renal disease and cataracts. The precise clinical presentation can be predicted based on the location of the mutation in *MYH9* [43].

Myosin XVA is the fourth unconventional myosin in hair cells known to cause hearing loss in humans. The protein localizes to the tips of stereocilia near the barbed ends of actin filaments, and extends into the apical plasma membrane [44]. Because it appears mainly at the developmental stage before the ‘staircase’ morphology of the hair bundle becomes apparent, myosin XVA may be involved in stereocilia elongation and formation. Studies of the mouse mutants shaker-2 (*Myo15a* mouse mutant) and whirler (*Whrn* mouse mutant) have shown that myosin XVA interacts with whirlin and moves it to the stereocilia tip links. Myosin XVA has a critical function in establishing the macromolecular complex between whirlin and two stereocilia-expressed proteins MPP1 (p55) and EPB4 (4.1R) at tip links [45]. In humans, 28 identified *MYO15A* mutations cause severe-to-profound ARNSHL, mainly in Pakistani consanguineous families [46].

Myosin IIIA is an unconventional myosin of the inner ear that causes hearing loss in humans. The protein is found at the tips of developing stereocilia surrounding the tip density region, a newly identified molecular compartment of stereocilia tips that may be the site of actin polymerization and operation of the MET apparatus. Myosin IIIA is also found further down the shaft of the stereocilia [47]. Although its function is not known, three possibilities have been proposed. First, myosin IIIA may be involved in stabilization of stereocilia length and tip shape; second, it may transport and assemble tip link complexes; or third, it may be involved in MET adaptation. In contrast to most other recessive mutations, *MYO3A* mutations cause late-onset, progressive ARNSHL that starts during the second decade [48]. To explain this unusual phenotype, it has been proposed that myosin IIIB partly compensates for lack of myosin IIIA [47].

Scaffolding proteins

Whirlin is an important scaffolding protein in the USH protein complex and links many different proteins. It is transiently expressed in stereocilia tips during elongation in both inner and outer hair cells and is also found at the base of stereocilia. The complex that forms between whirlin and myosin XVA may indirectly regulate actin polymerization and in this way, contribute to stereocilia elongation. Whirlin also directly interacts with USH2A and VLGR1b, and together with vezatin, these proteins form the ankle-link complex. There appear to be no direct interactions between whirlin and myosin VIIA [36]. In the recessive mouse mutant whirler (*wi*), *Whrn* mutations cause deafness and vestibular dysfunction due to impaired stereocilia elongation [49]. In humans, *WHRN* mutations cause profound ARNSHL and Usher syndrome type IID [50].

Harmonin is a scaffolding protein encoded by *USH1C*. Differential expression of alternatively spliced isoforms occurs. Isoform b is found in several tissues, including differentiating hair cells where concentrations are highest near the apex of maturing stereocilia. Other harmonin isoforms are found in stereocilia, the cuticular plate, the lateral plasma membrane and synapses [7]. The protein contains several important domains for protein-protein interactions including PDZ domains, coiled-coil domains and a PST (proline, serine, threonine-rich) domain for actin-binding. Two recessive mouse mutants, deaf circler (*dfcr*) and deaf circler 2 Jackson (*dfcr-2J*), carry *Ush1c* mutations and show deafness and circling behaviour [51]. Studies of *dfcr* indicate that harmonin b is essential for stereocilia development. The protein also plays an important role in MET [6]. As a scaffolding protein, it is essential to coordinate the organization of signal molecules in macromolecular protein complexes such as the Usher protein complex. For several reasons, cadherin 23 and harmonin are thought to act in a common molecular pathway. Both proteins can bind to each other mainly through PDF domains, they are coexpressed in the stereocilia, and mutations in the human genes *CDH23* and *USH1C* cause a similar Usher phenotype. Mutations in *USH1C* also cause ARNSHL at the DFNB18 locus. Whether the phenotype is one of nonsyndromic deafness or deafness and retinitis pigmentosa (USH syndrome) depends on the expression pattern and splicing of the different *USH1C* isoforms. Mutations causing USH syndrome are all truncating and occur in constitutive exons present in both the eye and cochlea. Missense mutations in alternatively spliced exons cause nonsyndromic hearing loss, as these exons are absent in the eye [52].

SANS is a scaffolding protein which interacts with harmonin, myosin VIIA and other SANS proteins through different protein domains [53]. The protein is concentrated beneath the stereocilia and may regulate vesicular trafficking of USH1 proteins to the stereocilia. As SANS is found in the synaptic region of hair cells, it may also take part in organization and/or function of hair cell synapses. Mutations in *SANS* have been found to cause Usher syndrome type 1G but have not been identified as a cause of nonsyndromic HL.

Extracellular matrix proteins

The sensory epithelia of the inner ear lie between two extracellular matrices: the basement membrane (BM) and the tectorial membrane (TM) (Fig. (2)). The latter is an acellular gel that overlies the surface of the organ of Corti. It consists of collagen fibrils embedded in a striated-sheet matrix. Only the tallest row of stereocilia of each outer hair cell is embedded in the TM. When sound waves cause a movement of the basilar membrane, the apical surface of the sensory epithelium will move towards the overlying TM. This movement deflects the stereocilia bundle, thereby modulating the gating of MET channels. Two genes, *TECTA* and *COL11A2*, have been identified as components of the TM, and mutations in these genes cause hearing loss in humans. Other components of the TM are otogelin and β -tectorin. Both genes are essential for normal auditory function in mice but have not yet been identified as a cause of deafness in humans [54,55].

α -tectorin is one of the major non-collagenous components of the tectorial membrane in the cochlea and the otolith membrane in the maculae of the vestibular system. The protein, which is mainly expressed during development of the TM, has an entactin G1 (ENTG1) domain, a central region with von Willebrand factor (vWF) type D repeats similar to zonadhesin (ZA) domain, and a single zona pellucida (ZP) domain. Through the ZA and ZP domains, α -tectorin is believed to interact with itself and with other extracellular matrix proteins including β -tectorin and several collagens [56]. Families with both ADNSHL and ARNSHL have been reported to carry mutations in *TECTA*. Dominant *TECTA* mutations can cause mid-frequency, high-frequency or progressive HL. The HL phenotype depends on the protein domain in which the mutation occurs and on the type of amino acid substitution [57]. ARNSHL-causing *TECTA* mutations also have a recognizable audioprofile characterized by moderate-to-severe

hearing loss across all frequencies. The HL is typically more pronounced in the mid-frequencies, resulting in a 'bowl'-like shape [58]. As this profile is distinguishable from most other ARNSHL phenotypes, it is an important clue for genetic diagnostics.

Type XI collagen A2 (*COL11A2*) is another component of the TM and is essential for maintaining the interfibrillar spacing and fibril diameter of type II collagen. Type II collagen is composed of three α -chain polypeptide subunits ($\alpha 1$, $\alpha 2$ and $\alpha 3$), each transcribed from a different gene (*COL11A1*, *COL11A2* and *COL2A1*). Mutations in *COL11A2* cause ADNSHL and ARNSHL in addition to different forms of osteochondrodysplasia such as Stickler syndrome. The observed phenotype depends on mutation type and location, as well as on modifying factors. Mutations in the gene probably cause hearing loss by changing the arrangement of collagen fibrils from an ordered parallel array to a more random pattern. The secondary consequence is an alteration of the mechanical properties of the TM [59]. ADNSHL caused by *COL11A2* mutations mainly affects the mid frequencies. Together with dominant *TECTA* mutations, these are the main dominant genes reported to cause mid-frequency HL.

Other components of the extracellular matrix are stereocilin, otoancorin and cochlin. The acellular gels of the inner ear are attached to underlying sensory epithelia, which consist of both sensory hair cells and nonsensory supporting cells. Two proteins, stereocilin and probably also otoancorin (encoded by *STRC* and *OTOA*, respectively), are present at the interface between the apical surface of the sensory epithelia and the overlying acellular gels of the cochlea and vestibular system [60,61]. These proteins are GPI-linked membrane-anchored proteins that have high sequence similarity at their C-termini. Both proteins may mediate the attachment of acellular gels to the epithelia but they act at different levels. Stereocilin may mediate the attachment of acellular gels to the sensory cells, while otoancorin may link nonsensory cells to the acellular gels. Mutations in both *STRC* and *OTOA* have been identified as a cause of ARNSHL [60,62].

Cochlin is an extracellular matrix protein found in the spiral limbus and spiral ligament. It has a ribbon-like distribution in the basilar membrane, suggesting its involvement in membrane structure and function [63]. Cochlin contains multiple domains including a short predicted signal peptide (SP), an N-terminal factor C homology (FCH or LCCL) domain, and two von Willebrand factor A-like domains (vWFA1 and vWFA2). Mutations in *COCH* cause a progressive form of ADNSHL with vestibular dysfunction. The phenotype is uniform and recognizable, which is useful for DNA diagnostics [64]. One mutation, P51S, is especially frequent in Belgian and Dutch patients due to a founder effect [65].

Ion homeostasis

Gap junctions are abundantly present in the cochlea and enable diffusion of small molecules between interconnected cells. Two distinct cochlear gap junction systems have been described: the epithelial and the connective gap junction system. The former is present between the nonsensory epithelial cells from the outer sulcus to the interdental cells, although there are no gap junctions between hair cells and supporting cells. The second network links the connective tissue cells of the spiral limbus and spiral ligament [66]. Together, these networks are believed to be involved in electrical and metabolic coupling in the cochlea.

The cochlea contains two major compartmentalized fluids, endolymph and perilymph, which differ in ionic composition. Perilymph is similar to other extracellular fluids and has a high Na^+ and a low K^+ concentration. In contrast, endolymph more closely resembles cytosol, with a high K^+ concentration and a low Na^+ concentration. The endolymphatic space also has a positive potential, the endocochlear potential.

As potassium ions enter hair cells in response to acoustic stimuli, a depolarization wave occurs which causes Ca^{2+} influx and neurotransmitter release. Electrical coupling facilitates ion homeostasis, in part by transport of potassium ions through gap junctions and potassium channels [67] (Fig. (2)). The involvement of gap junctions in metabolic coupling has also been shown by Beltramello and colleagues (2005). They observed that mutant C $\times$ 26 reduces metabolic coupling mediated by the cell-signalling molecule IP_3 (inositol 1,4,5-triphosphate) and impairs Ca^{2+} -wave propagation [68]. Several different genes are involved in inner ear ion homeostasis and have been linked to deafness. Examples include the connexins *GJB2*, *GJB3* and *GJB6*, ion channels *KCNQ4*, *SLC26A4* and *SLC26A5*, tight junctions *TRIC* and *CLDN14*, and other genes such as *TMPRSS3* and *CRYM*.

Connexins

Connexins are transmembrane proteins that form hexameric hemichannels or connexons. Connexons of adjacent cells couple to form functional gap junctions, which can be composed of connexons of one (homomeric) or different types (heteromeric) [69]. Connexin 26 (c $\times$ 26), connexin 31 (c $\times$ 31), connexin 30 (c $\times$ 30) and presumably also connexin 43 (c $\times$ 43) form gap junctions in the inner ear and mutations in the corresponding genes, *GJB2*, *GJB3*, *GJB6* and *GJA1*, are responsible for both syndromic and nonsyndromic forms of HL. C $\times$ 26 and C $\times$ 30 are the predominant isoforms expressed in cochlear supporting cells and are present in both the epithelial and connective tissue gap junction networks. Murine C $\times$ 31 is mainly expressed in type II fibrocytes of the spiral limbus and the spiral ligament, but is also seen in the auditory nerve [70]. C $\times$ 43 is expressed in supporting cells and type I fibrocytes [71].

Recessive *GJB2* mutations are a major cause of ARNSHL, accounting for about half of all cases of moderate-to-profound congenital deafness. It is estimated that at least 220 different mutations have been identified in the gene of which some are very frequent and some are rare (Connexin-deafness homepage: <http://davinci.crg.es/deafness/>; N.H., unpublished results). The 35delG mutation accounts for about 70% of *GJB2* mutations in many populations, with carrier frequencies up to 1 in 28 in the Mediterranean region [72]. A large genotype-phenotype correlation study has shown that HL caused by inactivating mutations in *GJB2* is more severe than HL caused by non-inactivating mutations [73]. Functional studies have demonstrated that most C $\times$ 26 mutant proteins cannot form functional gap junction channels *in vitro* and therefore perturb ion homeostasis. However, not all deafness-causing *GJB2* mutations result in complete loss of activity, suggesting that some mutations may interfere with permeability of other molecules such as IP_3 , which is critical for calcium wave propagation [68].

Most dominant *GJB2* mutations have been reported in Caucasians and about half of them are associated with nonsyndromic HL. The other mutations cause HL associated with heterogeneous skin disorders such as Keratitis–Ichthyosis–Deafness (KID) syndrome, Vohwinkel syndrome and diffuse palmoplantar keratoderma-hyperkeratosis [74]. Most dominant mutations occur in the first and second extracellular domains of the protein, which regulate connexon-connexon interactions. These mutations therefore alter either connexon composition, permeability or regulation [75]. In the skin, gap junction intercellular communication (GJIC) mediated by connexin signalling is believed to play a crucial role in the control of morphogenesis, differentiation and growth [76]. The dominant mutations that result in HL as well as in skin disorders probably not only cause a loss of C $\times$ 26 channel activity but also impact additional connexin genes thereby altering skin homeostasis and leading to disease. Consistent with this hypothesis, it has been proven that C $\times$ 26 mutants causing the skin syndromes exert a trans-dominant effect on C $\times$ 43 in the skin [77].

Mutations in *GJB6* have been implicated as the cause of hearing loss in many studies although only one mutation (T5M) is associated with ADNSHL [78]. The two large ARNSHL-causing deletions, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), occur upstream of *GJB2* and

truncate *GJB6*, but because they abolish *GJB2* expression, they should also be considered as *GJB2* mutations [79,80].

GJB3 mutations cause ADNSHL and ARNSHL as well as the skin disorder erythrokeratoderma variabilis. These mutations are an infrequent cause of hearing loss. One dominant mutation has been identified as the cause of auditory neuropathy and two others cause high-frequency hearing loss at the DFNA2 locus [70,81]. *GJB3* mutations causing ARNSHL have been reported once in two families in which patients were compound heterozygous for two different *GJB3* mutations [82]. The evidence that the identified variants in *GJB3* are disease-causing in these families is not compelling, making the role of *GJB3* as a nonsyndromic deafness gene questionable.

Mutations in *GJA1* have been reported to cause ARNSHL in four African-American families [71]. However, these results were later invalidated when the mutations were shown to involve only the pseudogene of *GJA1* on chromosome 5. *GJA1* mutations do cause oculodentodigital dysplasia (ODDD), a phenotypically variable autosomal dominant syndrome characterised by craniofacial and limb dysmorphology, spastic paraplegia and neurodegeneration. In some cases, syndactyly type III and conductive hearing loss are observed [83].

Ion channels

KCNQ4 is a member of the KCNQ family of potassium channels and is expressed in inner ear hair cells and the spiral ganglion. The KCNQ4 protein consists of six transmembrane domains and a P-loop region that forms the potassium-selective channel pore [84]. The precise role of KCNQ4 in hearing is not known. It was initially proposed that KCNQ4 may contribute to basolateral K⁺ conductance, important in the modulation of electrical excitation and removal of hair cell intracellular K⁺ [84]. However, more recent studies suggest that the progressive hearing loss may reflect dysfunction of both inner hair cells and spiral ganglion neurons, impairing electrical signalling [85]. Mutations in *KCNQ4* cause ADNSHL with a variable phenotype, explained by a genotype-phenotype correlation. *KCNQ4* missense mutations exert a dominant-negative effect with a mutant protein which interferes with the channel subunit and causes progressive hearing loss with an early onset. Both identified deletions in the gene act through haploinsufficiency, thereby causing a milder phenotype with later onset [86].

Pendrin, encoded by *SLC26A4*, is a transmembrane anion exchanger that belongs to the solute carrier 26 family and exchanges chloride, iodide, bicarbonate and formate. It is expressed in different tissues, including thyroid, kidney and inner ear. In the cochlea, it is found in the apical membrane of outer sulcus and spiral prominence epithelial cells that border the endolymph, in the spiral ganglion and in supporting cells [87]. Mutations in *SLC26A4* cause both Pendred syndrome (congenital HL, goiter and Mondini dysplasia) and ARNSHL with an enlarged vestibular aqueduct (EVA). It is often a challenge to distinguish between both phenotypes, as some of the symptoms such as goiter may occur at later age [88]. In addition, mutations in a transcriptional regulatory element of *SLC26A4*, as well as mutations in the transcriptional activator *FOXJ1* can also cause Pendred syndrome and ARNSHL [89]. The mechanism by which the loss of pendrin causes hearing loss has been clarified by studying mice homozygous for the targeted deletion of *Slc26a4*. In these mice, enlargement of the endolymphatic space and acidification of the endolymph are observed, which are believed to increase oxidative stress in the stria vascularis. Free radical stress causes reduced expression of *Kcnj10* and a subsequent reduction in the endocochlear potential, which is normally generated by this K⁺ channel. The reduced potential disturbs mechanotransduction and causes HL in these mice [90].

Prestin, encoded by *SLC26A5* is another member of the carrier 26 family of anion exchangers. The protein is expressed abundantly in the outer hair cells and plays a key role in their somatic electromotility (voltage-dependant cell-length variation). Variations in the outer hair cell

plasma membrane potential cause conformational rearrangements of prestin, which drive cellular contraction and elongation movements [91]. A homozygous mutation in *SLC26A5* has been identified as the cause of ARNSHL in two probands. Surprisingly, the same mutation has been found in the heterozygous state in seven patients with varying degrees of hearing loss, which suggests an interaction of *SLC26A5* with additional modifier genes [92].

Tight junctions

In the inner ear, tight junctions (TJ) separate the endolymphatic space from the perilymphatic space as both fluid-filled compartments have a different ionic composition. The bicellular tight junctions (bTJ) create a semipermeable paracellular seal between the apical membranes of the sensory hair cells and supporting cells. Tricellular tight junctions (tTJ) occur where three cells join together. TJs are composed of different molecules, including occludin, claudins and tricellulin.

Claudins are tetraspan integral membrane proteins that contribute to charge and size-selective pores of the paracellular barrier. Claudin 14 is one of the members of the claudin family which is expressed in different tissues including hair cells and supporting cells in the inner ear, sensory epithelium of the vestibular system, liver and kidney. The tight junction complex at the apex of the reticular lamina is believed to require claudin 14 as a cation-restrictive barrier to maintain proper ionic composition of the fluid around the basolateral surface of outer hair cells. In mice, mutations in *Cldn14* do not change the endocochlear potential but cause outer hair cell degeneration. *CLND14* mutations in humans cause profound congenital ARNSHL [93].

Another component of TJs is tricellulin, a tTJ protein, which plays a key role in the formation of barriers between tricellular contacts of epithelial cells throughout the body. It also may be a component of bTJs of epithelial barriers [94]. In the inner ear, the protein is present in tricellular junctions of the reticular lamina of the organ of Corti. Despite its ubiquitous expression, the only consequence of mutant *TRIC* alleles is moderate-to-profound ARNSHL. All mutations identified cause a truncation within the occludin-ELL domain, which is responsible for interactions with the scaffolding proteins ZO-1, ZO-2 and ZO-3. ZO-1 can interact with F-actin-linking transmembrane proteins to attach tight junctions to the actin cytoskeleton of the cuticular plate. Therefore, mutations in *TRIC* may cause hearing loss by reducing the rigidity of the reticular lamina [95]

Other proteins involved in ion homeostasis

Two possibly disease-causing mutations in *CRYM* (also called μ -crystallin) have been reported in two Japanese families segregating ARNSHL [96]. *CRYM* is identical to NADPH-dependent cytosolic T3 binding protein (p38CTBP) and is abundantly expressed in the vestibule and cochlea where it is distributed within type II fibrocytes of the spiral ligament and spiral limbus. As *CRYM* binds to T3, *CRYM* mutations may affect its binding properties with T3, thereby preventing transport of T3 from the cytoplasm to the nucleus of type II fibrocytes. These cells contain Na, K-ATPase β 1 subunits and are involved in ion homeostasis. As the promoter region of Na, K-ATPase β 1 has a functional T3 response element, the lack of T3 in the nucleus of fibrocytes may cause a dysfunction of the ATPase and disturb cochlear homeostasis [97].

WFS1 encodes for wolframin, a transmembrane protein with nine predicted helical transmembrane segments. Biochemical studies have shown that wolframin is an integral, endoglycosidase H-sensitive membrane glycoprotein expressed mainly in the endoplasmatic reticulum (ER). Its function in the inner ear is currently unknown, but it is believed to play a role in K⁺ and Ca²⁺ homeostasis maintained by the canalicular reticulum, a specialized form of ER. Wolframin is expressed in cells bordering the scala media, in the spiral ganglion and in vestibular hair cells. As the protein is expressed during all developmental stages, it could play

a role during the inner ear development or be involved in the maintenance of auditory function [98]. *WFS1* mutations cause both ADNSHL and Wolfram syndrome (ARHL, diabetes and optic atrophy). Dominant missense mutations in the C-terminal domain cause the very characteristic low frequency HL, while the high frequency thresholds are normal. At later age, the hearing loss progresses towards a flattened audioprofile. In contrast, inactivating mutations are the cause of the recessive Wolfram syndrome [99].

Transcription factors

EYA4 encodes for the Eyes-absent 4 protein. It belongs to the EYA family of transcriptional activators, which are part of a regulatory network of proteins essential for normal embryonic development. All EYA proteins contain a highly conserved *eyaHR* (*eya*-homologues) region and a transactivation domain called the *eyaVR* (*eya*-variable) region. Both *EYA1* and *EYA4* are expressed in the otic vesicle and have been implicated in human deafness as causes of branchio-oto-renal (BOR) syndrome and ADNSHL at the *DFNA10* locus, respectively. The *DFNA10* phenotype is a mid-frequency HL during childhood and adolescence that evolves to severe-to-profound HL across all frequencies by the sixth decade [100]. The four reported mutations occur in the *eyaHR* domain and are predicted to result in haploinsufficiency, suggesting that inadequate transcriptional regulation of the mature cochlea ultimately leads to HL [100]. Interestingly, one family has also been reported that segregates an *EYA4* mutation associated with HL and cardiomyopathy [101].

POU4F3 and *POU3F4* are two of 14 transcription factors in the POU superfamily. All members contain two DNA-binding domains, which bind a target DNA and are involved in tissue-specific gene regulation. *POU4F3* is a class IV POU protein and is essential for cell-specific maturation and survival. The gene is expressed in the cochlear and vestibular hair cells and regulates *Gfi1* and *Lhx3* [102]. It is probable that decreased expression of these two genes contributes to the molecular mechanisms underlying HL in the three families that have been identified with *POU4F3*-related deafness [103]. The segregating mutations affect DNA binding properties and cause a partial mislocalization outside the nucleus. The phenotypic consequence is HL that is highly variable in age of onset, level of progression and even audioprofile. In general, however, mid and high frequencies are affected with moderate-to-severe hearing loss [104].

POU3F4, also called Brain-4, belongs to class III POU proteins. The murine orthologue, *Brn4*, is expressed in periotic mesenchyme and may function with *Tbx1* to modulate gene expression in a critical subset of periotic mesenchyme cells on the ventral border of the otic vesicle. Interactions between these cells and otic vesicle epithelium may be necessary for proper cochlea formation and cell survival [105]. Mutations in *POU3F4* cause X-linked nonsyndromic HL at the *DFN3* locus. The HL may be mixed or purely sensorineural and is associated with defects in the bony labyrinth of the inner ear, including Mondini dysplasia and cochlear hypoplasia. Stapes fixation is also observed. However, corrective surgery is not recommended in these patients because of the risk of a perilymphatic gusher during surgery. Missense mutations in *POU3F4* localize to one of the two DNA-binding domains, although deletions of a putative regulatory element about 900 kb upstream of the gene also cause *DFN3*-related HL. No clear genotype-phenotype correlation is present [106].

Another putative transcription factor associated with ADNSHL is *TFCP2L3*, which shows sequence similarity to the TFCP2 family of proteins that bind many different gene promoters. In mice, *Tfcp2l3* is expressed in several epithelial tissues including epithelial cells lining the cochlear duct. While its function remains unknown, the observation that *TFCP2L3*-related HL starts at a later age suggests that the protein plays an essential role in maintenance of epithelial

cells. In addition to the single reported family that segregates *TFCP2L3*-related ADNSHL, the gene has been associated with presbycusis [107].

ESRRB encodes for the estrogen-related receptor protein beta, a member of the nuclear hormone receptor family of transcription factors. These proteins share a zinc finger DNA-binding domain and a ligand-binding domain. *ESRRB* is expressed in the spiral limbus, supporting cells, Reissner's membrane, stria vascularis, spiral ligament, nerve fibers and spiral ganglion cells, but it notably absent from sensory cells. Studies in mice homozygous for the targeted deletion of *Esrrb* have confirmed that the protein is essential for the development of marginal cells and a functional stria vascularis, as evidenced by disturbed endolymph production, aberrant inner-ear fluid homeostasis, and HL in these mutant mice. In humans, it is likely that *ESRRB* is required for these processes. *ESRRB* mutations lead to severe-to-profound ARNSHL [108].

Genes with poorly understood function

TMPRSS3 is a member of the Type II Transmembrane Serine Protease family (TTSP), a class of membrane-bound proteolytic enzymes that mediate a variety of biological processes. The gene has been implicated in cancer biology and also has an important role in the auditory system. It is expressed in the neuron bodies of the spiral ganglion, the stria vascularis and the epithelium of the organ of Corti. While the function of the protein is unknown, a role in MET is possible through regulation of ENaC (amiloride-sensitive) sodium channel activity and therefore cochlear sodium concentration. Several mutations have been described that cause ARNSHL. Most affected persons have severe-to-profound HL, but age of onset, severity and rate of progression are variable and no genotype-phenotype correlation has been established [109].

TMC1 or transmembrane channel-like gene 1 is a member of a new gene family of proteins. It contains several TM domains and charged intracellular amino and carboxy termini reminiscent of voltage-gated channels. Studies of the murine recessive mutant *deafness (dn)* and the dominant mutant Beethoven (*Bth*) have shown that protein expression begins before the onset of hearing in the pericuticular necklace and the ER of mature hair cells, suggesting a role for Tmc1 in normal hair cell maturation. Possibilities include the up- or down-regulation of ion channels or a role in intracellular trafficking [110]. Mutations in human *TMC1* cause both ARNSHL and ADNSHL. The recessive mutations all cause severe-to-profound HL. The dominant mutations have been reported in two North-American families; both families segregated mutations at amino acid position 572, suggesting that this amino acid position may be a mutational hot spot [111].

MYO1A, also known as the brush border myosin-I, belongs to the myosin superfamily. Members of this family have been implicated in various motile processes such as organelle translocation, ion-channel gating and cytoskeletal reorganization. All myosin I proteins share three structural domains: an ATP- and actin-binding head domain, a tail domain to target the proteins to specific subcellular locations, and a neck domain that connects them to myosin light chains. At the brush border surface of intestinal epithelial cells, myosin Ia is a major component of the actin-rich cytoskeleton, where it is involved in membrane trafficking. Myosin Ia could have a similar function in the inner ear, as the cytoskeleton of the intestinal cells and the inner ear cells (hair cells and supporting cells) are very similar. The specific expression pattern in the inner ear has not been established but may help to elucidate its function. Mutations in *MYO1A* have been implicated in ADNSHL in eight unrelated patients from Italy. All patients show a bilateral hearing loss of variable degree, which usually ranges from moderate-to-severe but is never profound [112].

MYH14 encodes for the non-muscle myosin heavy chain 14, which belongs to the conventional myosins of class II. High expression is found in the organ of Corti, cochlear duct and stria vascularis, while low-to-no expression is found in Reissner's membrane and the spiral ligament. This pattern differs from the myosin IX pattern, although both proteins belong to the same myosin group, suggesting a distinct function for each protein in the inner ear. Although the function of MYH14 has not been established, nonmuscle myosins participate in motility, cytokinesis, phagocytosis, maintenance of cell shape, and organelle/particle trafficking. *MYH14* mutations cause ADNSHL for which a genotype-phenotype correlation may be present: persons with nonsense mutations show profound HL, while persons with missense mutations have HL which is usually moderate-to-severe and variable in age of onset [113].

Two deafness genes of unknown function, *DFNA5* and *PJVK* (*DFNB59*), belong to the novel gasdermin protein family, of which all members contain a gasdermin domain. Both genes show significant sequence similarity. *DFNA5* is expressed in the epithelial ridge and stria vascularis of the developing inner ear. Although the gene is also expressed in many other tissues, persons with *DFNA5*-related ADNSHL have a phenotype that is limited to HL. This observation suggests that cochlear cells are very sensitive to the deleterious gain-of-function mutation that is characteristic of *DFNA5* deafness. All four reported *DFNA5* mutations are different at the DNA level but cause specific exon 8 skipping at the mRNA level, which leads to a truncated protein. The gain-of-function hypothesis is supported by three facts. First, human mutant *DFNA5* exerts a toxic effect on both yeast and mammalian cells [114], second, a mutation in exon 5 of *DFNA5* has been described that does not cause HL [115] and third, the HL in all four families with *DFNA5* mutations is very similar. It is progressive, starts in the high frequencies and evolves to become more severe with age. Although this phenotype resembles that of presbycusis albeit at a younger age of onset, no strong associations between age-related hearing impairment and *DFNA5* variants have been found [116].

Pejvak, encoded by *PJVK*, is found in spiral ganglion neurons and may play a role in action potential propagation or intracellular trafficking. These possible functions have been suggested by the observation that the two missense mutations initially identified in *PJVK* cause auditory neuropathy (absence of an auditory brainstem response (ABR) in the presence of oto-acoustic emissions (OAEs)) in both humans and *Dfnb59* knock-in mice [117]. Another report, however, has described families with pejvak mutations and severe-to-profound HL without auditory neuropathy [118]. These differences may reflect the degree of HL at the time of testing or possibly the type of mutation, with truncating mutations impairing outer hair cell function and missense mutations leaving their function intact.

TMIE or transmembrane inner-ear expressed gene encodes for a protein which shows no similarities to other proteins. The study of the recessive mouse mutant spinner (*sr*) carrying a mutation in *Tmie* suggests that the gene is required during maturation of sensory cells and is involved in the development or maintenance of stereocilia bundles. As the stereocilia of outer hair cells of spinner mice are shortened, *Tmie* might influence actin filament dynamics in the normal hair bundle or alternatively play a role in the organization of cytoskeleton-membrane interactions in sensory hair cells [119]. Its exact location in the inner ear is unknown. In humans, mutations in *TMIE* cause autosomal recessive severe-to-profound hearing loss [120].

OTOF encodes for otoferlin, a member of the mammalian ferlin family of membrane-anchored cytosolic proteins. All ferlins contain six calcium-binding C2 domains and are involved in vesicle membrane fusion. In the inner ear, *Otof* expression is found in high levels in inner hair cells and in lower levels in outer hair cells. The protein is probably essential for exocytosis and neurotransmitter release at the inner hair cell ribbon synapse [121]. Mutations lead to defective synaptic vesicle fusion which impairs neurotransmission. Because of the lower levels of otoferlin in outer hair cells, these cells are less affected [122]. As a result, in mice homozygous

for the targeted deletion of *Otof*, outer hair cell function is preserved and the auditory pathway is functional, while inner hair cell synaptic exocytosis is abolished [121]. *OTOF* mutations in humans cause profound ARNSHL, initially being consistent with auditory neuropathy (preservation of outer hair cell function) but progressing towards a loss of both inner and outer hair cell function. The presence of auditory neuropathy is an important indication for genetic diagnostics.

Mitochondrial hearing loss

Mitochondria are cell organelles responsible for ATP synthesis through oxidative phosphorylation. They are also involved in other cell functions such as apoptosis and oxidative stress control. Mitochondria have their own DNA (mtDNA) that encodes for proteins of the mitochondrial respiratory chain and for components of the protein synthesis apparatus (22 tRNAs, 2 rRNAs and 13 mRNAs). Thirty-seven mitochondrial proteins and about 60 nuclear-encoded proteins are involved in oxidative phosphorylation. Mutations in these genes cause a broad spectrum of mitochondrial genetic diseases, which are usually multisystem disorders. However, a subset of mutations only causes abnormalities in the cochlea and leads to nonsyndromic HL. Mitochondrial mutations are the cause of nonsyndromic HL in less than 1% of children with prelingual deafness and in at least 5% of postlingual HL cases in Caucasians [123].

MITOMAP gives an overview of most mitochondrial mutations identified (<http://www.gen.emory.edu/mitomap.html>). MtDNA is transmitted through the maternal lineage and mutations can be inherited or acquired. Nonsyndromic mitochondrial HL is most often associated with mutations in *MTRNR1* (encoding the 12S ribosomal RNA) and several tRNA genes including *MTTS1* (tRNA^{Ser(UCN)}). The most frequent mutation is 1555G>A in *MTRNR1*, which also causes aminoglycoside-induced HL. HL induced or worsened by the use of aminoglycosides can also be caused by other *MTRNR1* mutations, an association that can be explained by the similarity between human 12S rRNA and its bacterial homologue 16S rRNA, the target of these antibiotics. Carriers of 1555G>A are susceptible to HL after treatment with aminoglycosides at doses not normally implicated in HL, but HL can also develop without aminoglycoside exposure. The HL phenotype varies and includes severe congenital HL, moderate postlingual progressive HL and completely normal hearing [123]. This phenotypic variation can be explained by the influence of modifier genes. Three modifier genes, *TFB1M*, *MTO1* and *GTPBP3*, have been identified and are encoded by nuclear DNA but are involved in mitochondrial RNA processing and translation [124]. *TFB1M* is directly involved in mitochondrial 12S rRNA methylation, while *MTO1* and *GTPBP3* are proposed to be part of the mitochondrial tRNA modification machinery.

Mutations in *MTTS1* are another cause of mitochondrial nonsyndromic hearing loss, but with a low penetrance. It has been suggested that the *MTTS1* mutations alone are insufficient to cause HL and that aminoglycoside exposure is the main modifying factor. In addition, it has been observed that HL has a much higher penetrance if a *MTTS1* mutation occurs in combination with the 1555A>G mutation in *MTRNR1* [125].

Conclusion

Tremendous successes have been achieved in identifying genes that cause monogenic hearing loss, mainly by using positional cloning strategies. Extensive research has elucidated the function and expression pattern of these genes and their proteins, vastly improving our knowledge of the auditory system in the normal, as well as in the diseased state. However, causative genes have been identified for less than half of all mapped loci, and little is known about the genetics of complex forms of HL. Continued targeted efforts to address these gaps

are crucial to our understanding of the physiology and pathophysiology of hearing. The direct translational benefit of gene identification is an improvement in genetic diagnostics. Ultimately, however, improved patient care can be expected through the development of better and/or new habilitation options for hearing loss.

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Abbreviations

ABR	auditory brainstem response
ARHL	age-related hearing loss
ADNSHL	autosomal dominant nonsyndromic hearing loss
ARNSHL	autosomal recessive nonsyndromic hearing loss
BM	basement membrane
BOR syndrome	branchio-oto-renal syndrome
Cx	connexin
dBHL	decibel hearing level
ENTG1	entactin G1
ER	endoplasmic reticulum
ERM	e-zrin/radixin/moesin
EVA	enlarged vestibular aqueduct
FCH	factor C homology
GJIC	gap junctional intercellular communication
HL	hearing loss
IHC	inner hair cell
KID syndrome	Keratitis–Ichthyosis–Deafness syndrome
MET	mechano-electrical transduction
mtDNA	mitochondrial DNA
OAE	otoacoustic emission
ODDD	oculodentodigital dysplasia
OHC	outer hair cell
PAB	parallel actin bundle
SP	signal peptide
TJ	tight junction
TM	tectorial membrane
tTJ	tricellular tight-junction
TTSP	type II Transmembrane Serine Protease

vWF	von Willebrand factor
ZA	zona adherens
ZP	zona pellucida

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Fig. 1.
The hair bundle of cochlear hair cells with genes involved in hair bundle morphogenesis

Fig. 2.

Cross section of the cochlea. The numbers on the figure are explained in Table 2, except for 23 (scala vestibuli), 24 (scala media) and 25 (scala tympani)

Table 1

All 46 nonsyndromic deafness genes categorized according to their function in the hearing process. For each gene, the corresponding protein, locus/loci and mouse models (if available) are listed.

Gene	Protein	Locus	Spontaneous mouse model	ENU mutant	Targeted mutant
Hair bundle morphogenesis proteins					
<i>Proteins of the cytoskeleton</i>					
<i>ACTG1</i>	γ-actin	DFNA20/26			
<i>DIAPH1</i>	diaphenous 1	DFNA1			
<i>ESPN</i>	Espin	DFNB36	Jerker (<i>je</i>)		
<i>RDX</i>	Radixin	DFNB24			<i>Rdx</i> ^{-/-}
<i>TRIOBP</i>	TRIO and filamentous actin binding protein	DFNB28			
<i>CCDC50</i>	Ymer	DFNA44			
<i>Adhesion proteins</i>					
<i>CDH23</i>	cadherin 23	DFNB12-USH1D	deaf-waltzer (<i>v</i>); waltzer mouse nigata (<i>v^{ng}</i>); waltzer Jackson (<i>Cdh23^{3v-1}</i>); <i>Cdh23^{3v-2L}</i> ; <i>Cdh23^{3v-11L}</i>	<i>Cdh23^{3v-8L}</i> ; <i>Cdh23^{3v-9L}</i> ; <i>Cdh23^{3v-10L}</i>	
<i>PCDH15</i>	protocadherin 15	DFNB23-USH1F	Ames waltzer (<i>av</i>); Ames waltzer Jackson (<i>Pcdh15^{av-1}</i>); <i>Pcdh15^{av-2L}</i> ; <i>Pcdh15^{av-3L}</i> ; <i>Pcdh15^{av-9b}</i>	<i>Pcdh15^{av-nmf19}</i>	
<i>TMHS (LHFPL5)</i>	lipoma HMGIC fusion partner-like 5	DFNB67	hurry-scurry (<i>hscy</i>)	hurry-scurry-2-Jackson (<i>Tmhs^{hscy-2L}</i>)	
<i>VLGR1b (GPR98)</i>	G protein-coupled receptor 98	USH2C	Mass [Fringes]		<i>Vlgr1</i> /del7TM; <i>Vlgr1</i> ^{-/-}
<i>USH2A</i>	Usherin	USH2A			<i>Ush2a</i> ^{-/-}
<i>Motor proteins</i>					
<i>MYO6</i>	myosin VI	DFNA22-DFNB37	Snell's waltzer (<i>sv</i>)		
<i>MYO7A</i>	myosin VIIA	DFNA11-DFNB2-USH1B	shaker-1 (<i>sh1</i>)	headbanger (<i>Hdb</i>)	
<i>MYH9</i>	myosin, heavy chain 9, non-muscle	DFNA17			<i>MYH9</i> ^{-/-}
<i>MYO15A</i>	myosin XVA	DFNB3	shaker-2 (<i>sh2</i>)		
<i>MYO3A</i>	myosin IIIA	DFNB30			
<i>Scaffolding proteins</i>					
<i>WHRN</i>	Whirlin	DFNB31-USH2D	whirler (<i>wi</i>)		

Gene	Protein	Locus	Spontaneous mouse model	ENU mutant	Targeted mutant
<i>USH1C</i>	Harmonin	DFNB18-USH1C	deaf circler (<i>dfcr</i>) and deaf circler 2 Jackson (<i>dfcr-2J</i>)		<i>Ush1c216A</i>
<i>SANS (USH1G)</i>	SANS	USH1G	Jackson shaker (<i>js</i>)		
Extracellular matrix proteins					
<i>TECTA</i>	α -tectorin	DFNA8/12-DFNB21			<i>Tecta^{dENT/ENT}; Tecta^{Y1807C}</i>
<i>COL11A2</i>	collagen, type XI, alpha 2	DFNA13-DFNB53-STL3			<i>Col11a2^{-/-}</i>
<i>STRC</i>	Stereocilin	DFNB16			
<i>OTOA</i>	Otoancorin	DFNB22			
<i>COCH</i>	Cochlin	DFNA9			<i>Coch^{-/-}</i>
Ion homeostasis proteins					
<i>Connexins</i>					
<i>GJB2</i>	connexin 26	DFNA3-DFNB1			<i>Cx26^{QnosCre}; Cx26^{R73W}</i>
<i>GJB3</i>	connexin 31	DFNA2			
<i>GJB6</i>	connexin 30	DFNA3-DFNB1			<i>Cx30^{-/-}</i>
<i>GJA1</i>	connexin 43				
<i>Ion channels</i>					
<i>KCNQ4</i>	potassium voltage-gated channel, KQT-like subfamily, member 4	DFNA2			<i>Kcnq4^{-/-}; Kcnq4^{dn}</i>
<i>SLC26A4</i>	solute carrier family 26, member 4	DFNAB4-PDS			<i>Pds^{-/-}</i>
<i>SLC26A5</i>	solute carrier family 26, member 5 (prestin)				<i>Slc26a5^{-/-}</i>
<i>Tight junctions</i>					
<i>CLDN14</i>	claudin 14	DFNB29			<i>Cldn14^{-/-}</i>
<i>TRIC (MARVELD2)</i>	tricellulin (MARVEL domain containing 2)	DFNB49			
<i>Others</i>					
<i>CRYM</i>	μ -crystallin				
<i>WFS1</i>	wolframin	DFNA6/14/38-WFS			
Transcription factors					
<i>EYA4</i>	eyes absent homolog 4 (Drosophila)	DFNA10			<i>Eya4^{-/-}</i>
<i>POU4F3</i>	POU class 4 homeobox 3	DFNA15	dreidel (<i>ddl</i>)		<i>Bmn-3c^{-/-}</i>

Gene	Protein	Locus	Spontaneous mouse model	ENU mutant	Targeted mutant
<i>POU3F4</i>	POU class 3 homeobox 4	DFN3			<i>Brn-4^{-/-}</i> ; Sex-linked fidgeted (<i>sif</i>)
<i>TFCP2L3 (GRHL2)</i>	grainyhead-like 2 (Drosophila)	DFNA28			
<i>ESRRB</i>	estrogen-related receptor beta	DFNB35			
Proteins with poorly understood function					
<i>TMPRSS3</i>	transmembrane protease, serine 3	DFNB8/10			
<i>TMC1</i>	transmembrane channel-like 1	DFNA36-DFNB7/11	deafness (<i>dn</i>)	Beethoven (<i>Bth</i>)	
<i>MYO1A</i>	myosin Ia	DFNA48			
<i>MYH14</i>	myosin, heavy chain 14	DFNA4			
<i>DFNA5</i>	DFNA5	DFNA5			<i>Dfna5^{-/-}</i>
<i>PJVK</i>	pejvakian	DFNB59			<i>Dfmb59^{gm1 Ugs}</i>
<i>TMIE</i>	transmembrane inner ear	DFNB6	spinner (<i>sr</i>) and circling mouse (<i>cir</i>)		
<i>OTOF</i>	otoferlin	DFNB9			<i>Otof^{-/-}</i>
Mitochondrial proteins					
<i>MTRVR1</i>	mitochondrially encoded 12S rRNA (12S rRNA)				
<i>MTTS1</i>	mitochondrially encoded tRNA serine 1 (UCN) tRNA ^{Ser(UCN)}				

Table 2

The expression pattern of all nonsyndromic deafness genes in different structures and cell types of the inner ear. The numbers in the table correspond to the numbers on Figure 2. The genes highlighted in gray have a low expression in the corresponding structure/cell.

Nr	inner ear structure/cell type	genes											
		CDH23	CLDN14	KCNQ4	MYH14	MYH9	OTOF	PCDH15	WFS1				
1	inner hair cell												
2	outer hair cell	CCDC50	CDH23	CLDN14	KCNQ4	MYH14	MYH9	PCDH15	WFS1			WFS1	
3	interdental cells	ESRRB	GJB2	GJB6	TFCP2L3	WFS1							
4	inner sulcus cells	CLDN14	ESRRB	GJB2	GJB6	TFCP2L3	TFPRSS3						
5	inner pillar cell	CLDN14	ESRRB	GJB2	GJB6	MYH14	TFCP2L3	TFPRSS3					
6	outer pillar cell	CLDN14	ESRRB	GJB2	GJB6	MYH14	TFCP2L3	TFPRSS3					
7	Deiters' cells	CLDN14	ESRRB	GJB2	GJB6	MYH14	SLC26A4	TFCP2L3	TFPRSS3	WFS1			
8	Hensen cells	ESRRB	GJB2	GJB6	MYH14	PCDH15	TFCP2L3	TFPRSS3	WFS1				
9	Claudian cells	ESRRB	GJB2	GJB6	MYH14	PCDH15	SLC26A4	TFCP2L3	WFS1				
10	spiral ligament	COCH	CRYM	ESRRB	GJB2	GJB3	GJB6	MYH14	MYH9	POU3F4	SLC26A4	WFS1	
11	spiral limbus	COCH	CRYM	ESRRB	GJB2	GJB3	GJB6	MYH14	MYH9				
12	stria vascularis	CCDC50	DFNA5	ESRRB	GJB2	GJB6	MYH14	TFCP2L3	TFPRSS3				
13	spiral ganglion	ESRRB	KCNQ4	MYH9	PCDH15	PJVK	SLC26A4	TFPRSS3	WFS1				
14	auditory nerve	CCDC50	ESRRB	GJB3									
15	Reissner's membrane	CDH23	ESRRB	MYH14	POU3F4	TFCP2L3	WFS1						
16	tectorial membrane	COL11A2	TECTA										
17	basilar membrane	COCH											
18	external sulcus cells	GJB2	GJB6	MYH14	PCDH15	SLC26A4	TFCP2L3	WFS1					
19	spiral prominence	MYH14	TFCP2L3	WFS1									
20	bony spiral lamina	POU3F4											
21	reticular lamina	CLDN14?	TRIC										
22	space between IDC and TM	OTOF	STRC?										
1 and 2	exclusive expression in inner and outer hair cells	ACTG1	ESPN	MYO15A	MYO3A	MYO6	MYO7A	POU4F3	RDX	STRC	TMC1	TMHS	
		TRIOBP	USH1C	WHRN									
	unknown expression pattern	DIAPH1	EYA4	MYO1A	TMIE								