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Hearing Loss: Mechanisms Revealed by Genetics and Cell Biology

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inner ear, deafness, cochlea, hair cell, mouse, mutation

Abstract

Hearing loss (HL), or deafness in its most severe form, affects an estimated 28 and 22.5 million Americans and Europeans, respectively. The numbers are higher in regions such as India and the Middle East, where consanguinity contributes to larger numbers of recessively inherited hearing impairment (HI). As a result of work-related difficulties, educational and developmental delays, and social stigmas and exclusion, the economic impact of HL is very high. At the other end of the spectrum, a rich deaf culture, particularly for individuals whose parents and even grandparents were deaf, is a social movement that believes that deafness is a difference in human experience rather than a disability. This review attempts to cover the remarkable progress made in the field of the genetics of HL over the past 20 years. Mutations in a significant number of genes have been discovered over the years that contribute to clinically heterogeneous forms of HL, enabling genetic counseling and prediction of progression of HL. Cell biological assays, protein localization in the inner ear, and detailed analysis of spontaneous and transgenic mouse models have provided an incredibly rich resource for elucidating mechanisms of hereditary hearing loss (HHL). This knowledge is providing answers for the families with HL, who contribute a great deal to the research being performed worldwide.

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INTRODUCTION

The exquisite sensory transduction of the cochlea is dependent on a tremendous amount of synchronized processes and mechanisms that require a large battery of protein-coding genes as well as regulatory elements. In the past two decades, while the Human Genome Project (HGP; **http://genomics.energy.gov**) was ongoing, extensive research on the genetics of hearing loss (HL) was performed worldwide, leading to the discovery of many genes essential for hearing. However, the cloning of the responsible genes was not enough, and broad studies on animal models were conducted in order to provide a better understanding of the function of each gene and its relation to the auditory network. The striking similarities between the human and mouse inner ears paved the way to the understanding of many processes and to the gathering of an enormous amount of information about this organ in a way that could not have been achieved by just exploring the human ear. In this review, we provide an overview of the human ear and hereditary hearing loss (HHL), a historical perspective of the research in this field, a description of protein families associated with deafness grouped by function, and the contribution of mouse models to our study of the pathology and etiology of hereditary hearing impairment (HI) in humans. Another useful model system for studying HL is the zebrafish, *Danio rerio*, which has been reviewed in recent years (111) and will not be further covered in this review. Understanding the mechanisms of the inner ear pathways, both in the functional and the pathological state, is enabling researchers to develop cell- and gene-based therapy treatments that may eventually be implemented in humans with HI.

Cochlea: a snail-shaped organ comprises the auditory apparatus of the inner ear; highly similar between human and mouse

HGP: Human Genome Project

HL: hearing loss

HHL: hereditary hearing loss

HI: hearing impairment

Cochlear duct: a complex of a coiled duct that reaches from the basilar toward the apical portion of the cochlea

Endolymph: the fluid inside the scala media

Perilymph: the fluid inside the scala tympani and scala vestibuli

Hair cells: sensory cells of the auditory system that reside in the sensory epithelium of the cochlea

The Complexity of Mammalian Inner Ear Structure

A mixture of sound waves is constantly generated in the environment and reaches our ears. This rich collection of sounds is scrambled and contains a wide spectrum of low and high tones in a variety of intensities. The ear is an outstanding organ that can precisely decipher between different frequencies and intensities (50), while simultaneously transferring the gathered information to the brain and enabling us to categorize each sound and learn about its relative distance and direction (71).

The ear is divided into three anatomical compartments: the outer, the middle, and the inner ear (**Figure 1*a***). The main role of the outer ear and the middle ear is to conduct the sound waves from the environment into the inner ear. The sound waves are captured by the outer ear auricle and conducted through the ear canal to reach the eardrum membrane. The middle ear is composed of three small bones, the ossicles, that are assembled together and serve as a link between the eardrum and the oval window of the fluid-filled inner ear. Upon sound stimulus, vibration of the eardrum will lead to a pistol-like movement of the middle ear bones and to movement of the fluids inside the inner ear, the sensory portion of the ear (97).

The inner ear plays two pivotal roles in life. It contains the sensory organs that enable hearing and that control our balance and spatial orientation. The concealed anatomical position of the inner ear inside the human head, deeply embedded in the temporal bone, provides a protected shield to this tiny, sensitive sensory organ. It also serves as an acoustic chamber that will support capturing even a very low intensity of sound. The auditory apparatus of the inner ear is composed of the cochlea, a snail-shaped organ surrounded by the temporal bone (**Figure 1*a***). It contains the cochlear duct that runs along the spiral shape from base to apex (**Figure 1*b***). This complex, coiled duct is divided by two thin membranes into three different compartments filled with fluids. The scala media is an endolymph-filled cavity located in between the scala vestibuli and scala tympani, perilymph-filled cavities. The scala media contains the organ of Corti, which is the sensory epithelium of the auditory system (reviewed in Reference 117). The organ of Corti sits on the basilar membrane that separates the scala media and scala tympani. Specialized sensory cells called hair cells are

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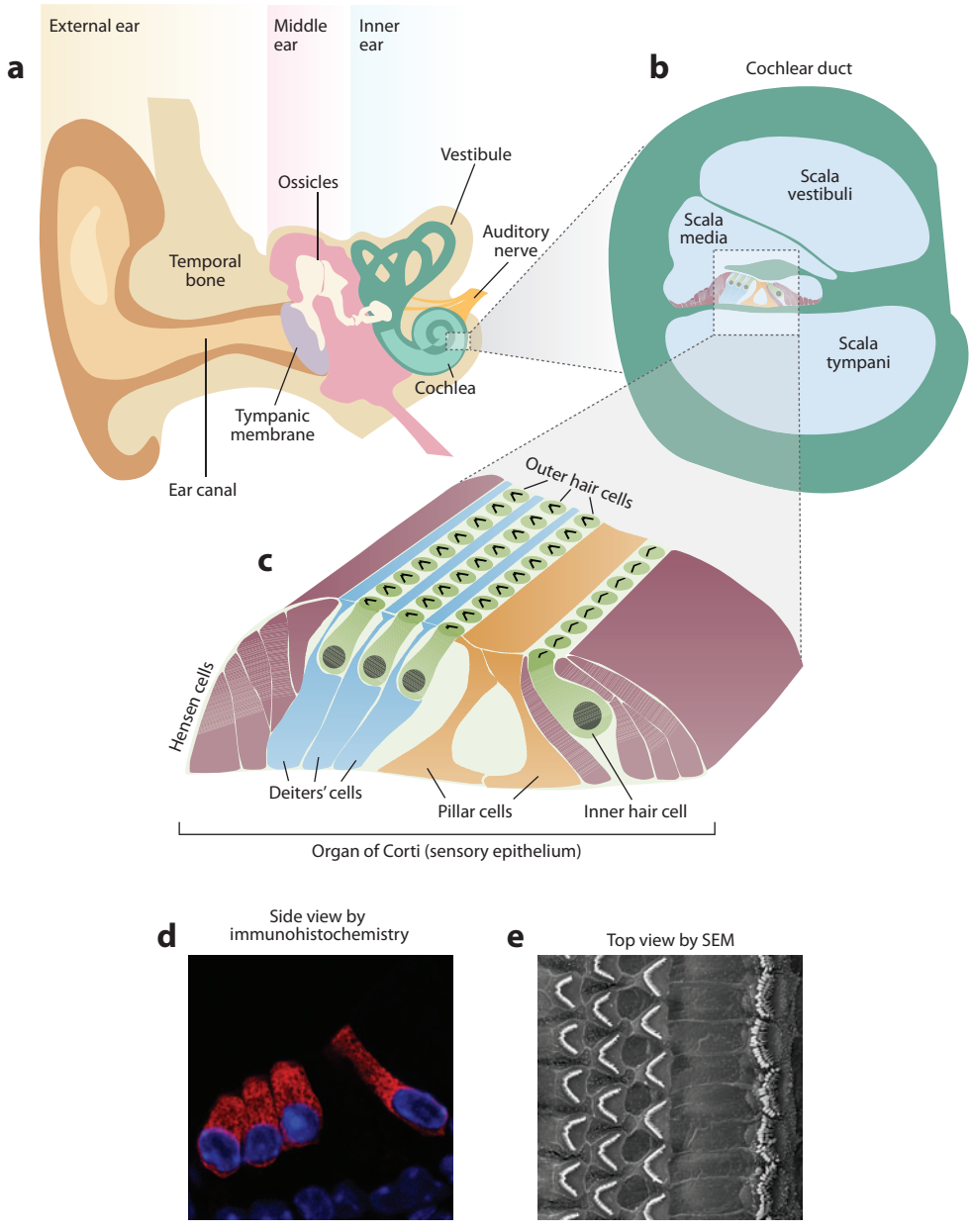

Figure 1

Schematic illustration of the human ear. (*a*) The ear consists of the outer, middle, and inner ear. (*b*) A section through the cochlear duct illustrates the fluid-filled compartments of the inner ear (modified from Reference 43). (*c*) The organ of Corti resides in the scala media, with sensory hair cells surrounded by supporting cells that include Deiters', Hensen, and pillar cells (modified from Reference 43). (*d*) Immunohistochemistry with the inner ear hair cell marker myosin VI, marking the cytoplasm of inner and outer hair cells, and 4′,6-diamidino-2-phenylindole (DAPI), marking the nuclei. (*e*) Scanning electron microscopy image of the top view of the sensory epithelium reveals the precise arrangement of one row of inner hair cells and three rows of outer hair cells, separated by the pillar cells. Figures designed and created by A. A. Dror.

Hair bundle: group of stereocilia organized in a typical staircase structure residing on the apical surface of the hair cells immersed in endolymph

embedded in this sensory epithelium, arranged in a highly organized conformation of one inner row and three outer rows of hair cells (**Figure 1*c–e***, **Figure 2*a***). The apical side of the hair cells facing the scala media contains actin-rich projections called stereocilia (**Figure 2*b***). The stereocilia have a typical staircase arrangement connected with lateral and tip links stabilizing the mature hair bundle structure (**Figure 2*c***; reviewed in References 36 and 45). The hair cells are covered with the tectorial membrane, a collagen-rich

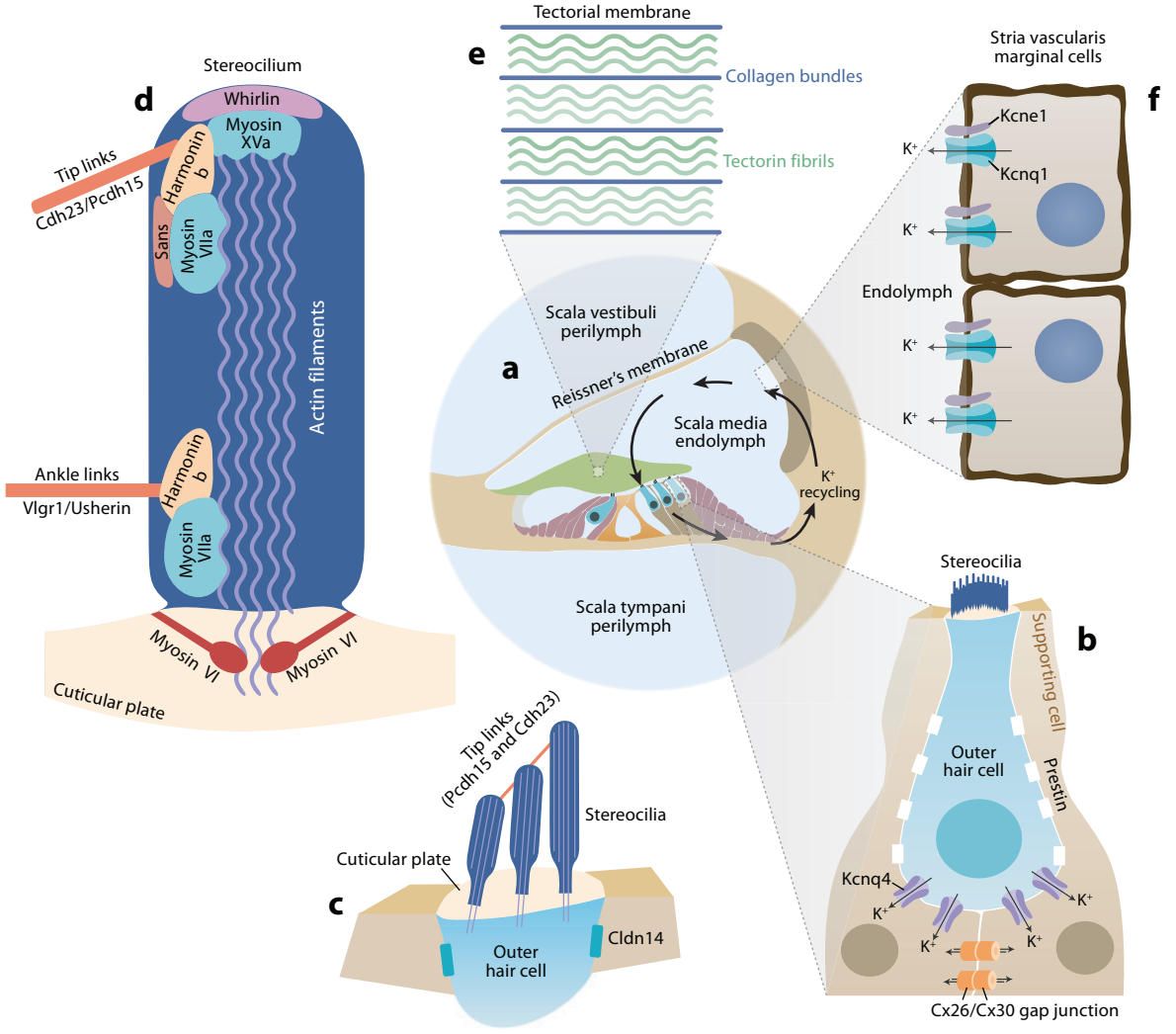

Figure 2

Schematic illustration of portions of the inner ear, with expression of some proteins that play a role in inner ear function in humans. Only some proteins are shown. (*a*) Cross section through one turn of a mouse cochlea. (*b*) Illustration of a single outer hair cell (*blue*), surrounded by two Deiters' supporting cells (*brown*). (*c*) Enlargement of the hair cell hair bundle with actin-based stereocilia (*blue*). (*d*) A single stereocilium containing actin filaments. (*e*) Illustration of the tectorial membrane that is composed of thin tectorin fibrils (*green*) and heavy collagen bundles (*blue*). (*f*) Marginal cells of the stria vascularis. (Modified from Reference 42). Figures designed and created by A. A. Dror.

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extracellular matrix (**Figure 2*e***; reviewed in Reference 120). When sound is induced, fluids move through the cochlear duct and vibrate the basilar membrane with the sensory epithelium against the tectorial membrane (**Figure 2*e***). These vibrations activate mechanoelectrical transduction, triggering the hair cells via deflection of the hair bundles and enabling potassium influx through the apical transduction channels that depolarize the cells. This sensory transduction is dependent on constant maintenance of the unique ionic composition of the endolymph. The strial marginal cells of the lateral wall of the scala media are one of the most important key players in this respect (**Figure 2*f***). The stria vascularis is responsible for secreting a constant supply of potassium into the endolymph as well as for generation of the endocochlear potential (reviewed in Reference 156).

As can be seen in the following descriptions, genes responsible for deafness have been discovered in each portion of the inner ear, and their study has provided us with a deeper understanding of the physiological and mechanistic aspects of each part.

Major Forms of Human Deafness

Given the complexity of the inner ear, it should come as no surprise that human HI is complicated as well. There are many forms of HI, both from a clinical and pathological point of view. HI is considered to be the most prevalent sensory disorder in humans, caused by a variety of genetics and environmental factors. The environmental factors include frequent exposure to high-intensity sound, acoustic trauma, viral infection, and ototoxic drugs, whereas the genetics factors are caused by mutations in different genes or regulatory elements that are involved in proper development, structure, and function of the ear. Nevertheless, the dichotomy between environmental and genetics factors is not always clear. Several studies have shown that mutations in some genes are associated with noise-induced hearing loss (NIHL), increasing the risk of affected individuals to lose their hearing under exposure to high-intensity sounds (74). Other common HL classifications are based on the affected portion of the ear, the age of onset, and the accompanied manifestations. The majority of the known genetic mutations affecting the inner ear components lead to sensorineural HL, whereas a smaller number are associated with middle ear conductive HL. Deafness loci are given the prefix DFN, followed by a number indicating the order in which they were discovered. The inheritance mode of the known genetic mutations that lead to deafness can be either autosomal recessive (DFNB), autosomal dominant (DFNA), X-linked (DFN), or mitochondrial. Some, like DFNA15/*POU4F3* (144), have been found in very few families in the world, and others, such as DFNB1/*GJB2*, occur almost everywhere in the world (Connexin-deafness homepage, **http://davinci.crg.es/deafness/**) (see sidebar, Online Resources and Databases). In most cases, there is only one known human deafness gene in the specific chromosomal region, as for *COCH* (DFNA9) (121). In other cases, there are two genes in one locus (e.g., *GJB2* and *GJB6* in DFNB1) (29, 65).

Approximately 50% of inherited HI cases are accompanied with other clinical features and are categorized as syndromic hearing loss (SHL) versus nonsyndromic hearing loss (NSHL), where HI is the only phenotype.

ONLINE RESOURCES AND DATABASES

Progress in the field of hereditary hearing loss (HHL) has been so rapid that the majority of scientists rely on the updates provided by a number of Web sites. Perhaps the most relevant one for the field has been the Hereditary Hearing Loss Homepage (**http://webh01.ua.ac.be/hhh/**). Gene-specific databases with user-friendly Web sites include the Connexin-deafness Homepage (**http://davinci.crg.es/deafness/**). Mouse models for hearing loss (HL) are highlighted at the Jackson Laboratory on the Hereditary Hearing Impairment in Mice site (**http://hearingimpairment.jax.org/**).

Mechanoelectrical transduction: conversion of mechanical forces of sound into electrical signals, achieved by deflection of the hair bundle

NIHL: noise-induced hearing loss

DFN loci: nomenclature for naming and numeration of chromosomal loci that are linked with hearing impairment

NSHL: nonsyndromic hearing loss

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SHL: syndromic hearing loss

SNL: syndromic hearing loss

PS: Pendred syndrome

ARHL: age-related hearing loss

Presbyacusis: age-related hearing loss

Consanguinity: relation that exists between persons, with one either descended from the other, or descended from the same common ancestor

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Interestingly enough, several genes harbor mutations leading to both SHL and NSHL, though at times controversies surrounding the potential lack of obvious syndromic symptoms exist. For example, mutations in myosin VIIa (*MYO7A*) occur in both USH1B and the nonsyndromic DFNB2. However, there are patients first diagnosed with the nonsyndromic form that later develop symptoms of USH1B (179). The original DFNB4 family (8), initially claimed to have NSHL, was subsequently found to have Pendred syndrome (PS). Later reports did, however, describe *PDS* mutations in NSHL (17, 90). In other cases, the demarcation between syndromic and nonsyndromic HL appears clear; for example, with cadherin 23 mutations, associated with USH1D and DFNB12 (12, 13).

Another common classification is based on the age of onset of the HL and distinguishes between prelingual and postlingual phenotypes, before or after language acquisition, respectively. Statistical data shows that one in every one thousand newborns suffers from profound HL and another one will become profoundly deaf before adulthood (99, 132). Furthermore, HI can arise later in age. More than 25% of 65 year olds and more than 50% of the population older then 80 suffer from different degrees of age-related hearing loss (ARHL), also known as presbyacusis. Extensive genetic linkage studies of genes for SHL and NSHL in families, performed by researchers all over the world, have yielded more than 46 genes identified to date (Hereditary Hearing Loss Homepage, **http://webh01.ua.ac.be/hhh/**). In addition to linkage of monogenic forms of HL, efforts are being made by genome-wide association studies to mine for genes for NIHL and ARHL. Identification of genes involved in proper hearing is critical to expand our understanding of how the ear works, to improve diagnosis, and to develop pioneering therapeutic approaches in the long run. In addition, it also allows for the option of genetic screening during pregnancy, although this is a controversial issue (18, 104), or at work places with constant exposure to high-intensity sounds.

History of Genetics of Human Deafness

Reports as early as the sixteenth century describing HHL were not favorable, as they advocated preventing the deaf from marrying as they would have deaf children themselves (reviewed in Reference 49). The nature of recessive and dominant HL was referred to already in the sixteenth and seventeenth centuries, with reports about families with consanguinity in the former case and otosclerosis in the latter case. A founder effect was also noted in reference to Martha's Vineyard, an island off the coast of Massachusetts in the United States, with the earliest recorded deaf person born in 1694 (52). Full social acceptance of the deaf was exemplified by the fact that all islanders, both hearing and deaf, learned and communicated in sign language. Already in 1882, the large prevalence of HL due to inheritance was noted by the statement: "the most frequent causes of congenital deafness are hereditary. . ." Over the years, many more reports appeared about HHL. One of the strongest advocates of preventing marriage among the deaf was none other than Alexander Bell, whose mother and wife were both deaf. Although Bell made a tremendous contribution to communication with his invention of the telephone, he proposed elimination of deaf schools, sign language, and government jobs for the unfit. Eugenics, which discouraged reproduction by people with genetic diseases, continued and had tragic consequences in Nazi Germany, where 1600 deaf were killed and 17,000 sterilized. Today, there are many schools of thought regarding education and language for the deaf and a strong deaf culture in some countries (reviewed in Reference 14). The deaf have a fascinating and complex culture. Most interesting is a theater group of deaf (and blind, many of whom suffer from Usher syndrome) actors and actresses, Nalaga'at Deaf-Blind Theater Company, who perform in a center that includes a restaurant with blind servers and a café with deaf servers (**http://www.nalagaat.org.il/en/**). Gallaudet University, located in Washington

DC, is the only institution of higher education for the deaf and hard of hearing (**http://www.gallaudet.edu/**).

Reports of HHL continued to appear, including one of a genetic form of deafness described in 1968 as "dominantly inherited low frequency hearing loss" (W. Nance, personal communication; 148), which turned out to be a dominant mutation at the *DFNA6/DFNA14* locus when the *WFS1* gene was identified (11, 173). In 1975, Nance & Sweeney (108) described the forms of HL, the need for research to detect carrier status, and the reality that genetic counseling was the only course available at that time. In 1988, the mapping of an X-linked form of deafness was accomplished in a large Mauritian kindred (153). It was seven years before the responsible gene, *POU3F4*, was discovered (28). In 1992, the first locus for NSHL was discovered with the mapping of the *DFNA1* locus in a Costa Rican kindred (88). The gene responsible, *diaphanous* (*DIAPH1*), was identified approximately five years later and found to encode a protein that regulates polymerization of actin (94). Subsequently, approximately 56 additional dominant loci have been found, although not all of their genes have been identified (Hereditary Hearing Loss Homepage, **http://webh01.ua.ac.be/hhh/**).

In 1994, the first recessive NSHL locus *DFNB1* was mapped (54). Estimates of the number of recessive deafness genes were quite low over the years, ranging from two to six genes (41). *DFNB1* turned out to be the most prevalent locus, first discovered in 1994 (54) and followed three years later by the finding of the gene connexin 26 (*GJB2*) (65). Today, the number of recessive genes stands at 26 and is still rising given that another 35 loci have been identified, although the corresponding genes are not yet known for this group (Hereditary Hearing Loss Homepage, **http://webh01.ua.ac.be/hhh/**).

For SHL, *USH2A* was the first locus mapped in 1990, which was later identified as the *USH2A* gene (37, 70). Subsequently, 32 genes for nine syndromic forms of HL have been discovered, including those for Norrie disease, Stickler syndrome, Treacher Collins syndrome, Alport syndrome, Branchio-Oto-Renal syndrome, and Waardenburg syndrome as well as those discussed in this chapter for Usher syndrome, Jervell & Lange-Nielsen syndrome, and PS (Hereditary Hearing Loss Homepage, **http://webh01.ua.ac.be/hhh/**).

An account of the history of genetics of HL cannot be given without describing the immense contribution provided by the mouse. An account of the circling or waltzing mouse is provided in a review by Ruben (126), where he describes the earliest known record from the Chinese Han dynasty in 80 BCE. Many years later, the Japanese collected mice as pets, most notably for their intriguing coat colors. Dancing/waltzing mice were already described then to be poor breeders, and today scientists working with homozygote circling mice try to refrain from using the females for mating. The Japanese had little interest in the circlers, but when the mice reached Europe in the nineteenth century, there was more of an interest in their circling behavior, and they were kept in large cages to observe this phenomenon. Yerkes, in his PhD thesis in 1902 and later in a book, described the behavioral and auditory abnormalities in mice originating from a mouse collector in Massachussets (172). These mice were later moved to Harvard for further study. In the early twentieth century, their value in studying the development and morphology of the ear became apparent. The deaf and circling *shaker1* (*sh1*) mutant was one of the first studied, and it is very appropriate that this mutant turned out to be one of the first deaf mice whose gene was cloned (48). The shaker1 mouse was the crucial factor for helping identify the *USH1B* locus (161). Over the years, many more mouse mutants were instrumental in the discovery of the human deafness gene (and vice versa). Most significantly, the development and morphology of inner ears of deaf mouse mutants was studied by many before genes arrived on the scene, including Malkiat Deol, who in 1956 described the pirouette, shaker1, and waltzer mice (32). In 1980, 22 years before its cloning, one of the few noncircling mutants,

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deafness (*dn*), was described in *Nature* (139). In fact, it was the discovery of both recessive and dominant forms of human deafness with *TMC1* mutations that led to the finding of the mouse gene (79, 152).

The Mouse as a Model for Human Deafness

With the completion of the mouse genome draft sequence, it became clear that the similarity between the human and mouse genomes is tremendous. Both have ~20,000 protein-coding genes, approximately 40% of the mouse genome can be aligned with the human genome at the nucleotide level, and above 90% of the mouse and human genome can be divided into parallel segments of synteny, conserving gene order originating from our common ancestor (23). The divergence of both species occurred roughly 90 million years ago, a short period in terms of evolution but long enough to generate changes at the DNA level, such as insertions, deletions, duplications, and polymorphisms. By using comparative genomics approaches, it is now possible to identify sequences that remained conserved during evolution. These conserved sequences may serve as clues to illuminate essential regulatory and other key elements. *Homo sapiens* and *Mus musculus* are both mammals, and therefore, they share many common systems in their bodies, as compared with other vertebrates. A good example for this similarity can be found in the HL field of research. The formidable similarities between the human and mouse inner ears paved the way to understand many processes and gather an enormous amount of information about this organ in a way that we could not have achieved by just exploring the human ear. Although most of the inner ear studies in humans are performed postmortem, in mice we can access the ear at any stage of development. Finally, and equally significant, the ability to manipulate the mouse to generate mutants, using gene-targeted mutagenesis or ENU (15), with inner ear defects has provided unparalleled conclusions about mechanisms of auditory function and human HL (For examples, see References 24, 30, 38, 43, 124, 151).

N-ethyl-N-nitrosourea (ENU): chemical mutagen

FUNCTION OF THE INNER EAR: FROM GENETICS TO CELL BIOLOGY

The discovery of human deafness genes has provided a tremendous amount of knowledge regarding the inner ear. However, in order to understand the mechanisms of HL and its associated pathology, cell biology was required for functional assays and gene and protein expression analysis. Together with genetics, cell biological studies supplied vital information about components of the inner ear. These include the cytoskeleton, cell-cell junctions, membrane transport, and regulatory elements, which are expanded upon in this review. There are other components that are covered, including the extracellular matrix and other structural components.

The Cytoskeleton

The cytoskeleton is composed of a network of protein filaments organized in the cell's cytoplasm (5). The ability of a cell to move, to have a particular shape, and to organize its internal components is dependent on this cytoskeleton. Three types of protein filaments form the framework for the cytoskeleton: intermediate filaments, microtubules, and actin filaments. Actin filaments are composed of helical polymers of actin and are polarized, with tightly regulated assembly/polymerization and disassembly/depolymerization of actin at each end. Motor proteins, including kinesins and dyneins that move along microtubules, and myosins that move along actin filaments, use the energy of ATP hydrolysis to move along the filaments. Although cytoskeletal proteins have similar functions in most cell types, the unique structure of the hair cells, with their actin-rich stereocilia, requires additional or different functions (reviewed in Reference 95). Stereocilia bundles have a characteristic size and shape (**Figure 3*a***), which is determined by the hair cell's location.

Stereocilia length is tightly regulated, with continuous actin turnover. They contain a rigid paracrystalline array of parallel and polarized cross-linked actin filaments. The rate of actin polymerization and depolymerization is dependent on a number of factors, including myosin motors.

A significant number of deafness genes encode proteins associated with the cytoskeleton in some fashion. These include myosins, outlined below. Mutations in *ACTG1*, which encodes a gamma isoform of actin, is associated with DFNA20/DFNA26 (147, 178). An inner ear-specific form of *TRIOBP*, coined "ototara," encodes an actin-binding protein. Associated with *DFNB28*, mutations have been found in both the Palestinian (135) and Pakistani (119) HI populations. As discussed previously, *DFNA1* is associated with diaphanous mutations, and the protein diaphanous homolog 1 is speculated to play a role in actin polymerization in the inner ear (94). Espins are actin-bundling proteins that have been shown to be directly involved in stereociliary growth and length maintenance by stabilizing actin filaments (127). Mutations in *espin* were first found in the jerker deaf mouse, leading to shorter and thinner stereocilia (**Figure 3*b***) (177), followed by the identification of human mutations (109). Radixin is an example whereby the phenotype in a mouse mutant led to the discovery of human mutations. Radixin-deficient mice were found to be deaf (72). Radixin is a member of the ezrin/radixin/moesin (ERM) protein family of the band 4.1 superfamily, which cross-links actin filaments to the plasma membrane. Screening of Pakistani families with markers from the region containing radixin, DFNB24, led to the identification of mutations in this gene (66). *Whirler* mouse mutants possess shorter than normal stereocilia (**Figure 3*c***) (106), and cloning of the gene indicated that it codes for a PDZ domain molecule involved in actin dynamics and stereocilia elongation (100). BAC transgenesis confirmed *whirlin* as the causative gene for the whirler mouse mutant phenotype. Subsequent sequencing of this gene in DFNB31 families, whose locus mapped to

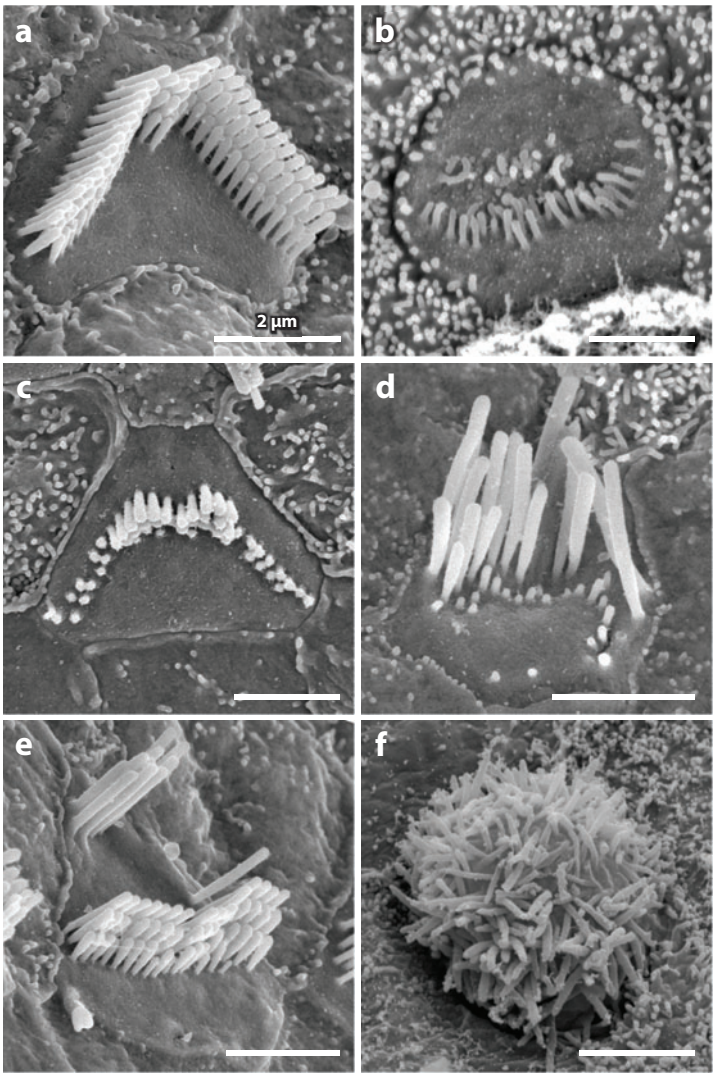

Figure 3

A sampling of cochlear outer hair cells from wild-type and mutant mice, demonstrating the effect that mutations in different proteins have on the morphology and function of hair cell stereocilia. High-resolution scanning electron microscopy reveals abnormal structures in different mutants. (*a*) A wild-type mouse at postnatal day 21 (p21). (*b*) The jerker mouse lacking espin at p12 has shorter and thinner stereocilia (127). (*c*) The whirler mouse lacking whirlin at p20 has shortened stereocilia (106). (*d*) A myosin VIIa-deficient mouse at p28 has longer stereocilia (115). (*e*) The Tailchaser myosin VI mutant mouse at p21 has branched and fused stereocilia (56). (*f*) A *Dicer-PCKO* mouse at p38, lacking microRNAs in the hair cells, has rounded hair bundles (43). Scale bars, 2 µm.

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the homologous mouse chromosomal region, led to the discovery of human mutations (100).

Mutations in the gene *SLC26A5*, encoding prestin, were found in recessive forms of HL (91). A member of the solute carrier family that encodes anion transporters, prestin functions as a motor protein specifically in outer hair cells. Most significantly, prestin is the molecular motor that performs electro-mechanical conversion, voltage-dependent mechanical changes in the outer hair cells required for amplification of sound (176). A mutation in the *CCDC50* gene in the DFNA44 family encodes Ymer, an effector of epidermal growth factor (EGF)-mediated cell signaling that may inhibit down-regulation of the EGF receptor (105). Based on its expression in the inner ear, it has been suggested that it plays a role in the microtubule cytoskeleton and would thus be the first protein implicated in this portion of the cytoskeleton.

Myosins. Myosins are a family of structural proteins and among the first proteins found to be associated with human deafness, via the mouse. Eighteen classes are defined by differences in their motor domain, with the similarities defining their homology within one family (reviewed in 19). They are actin-activated Mg^{2+} ATPases that move along actin filaments, using energy from ATP hydrolysis. The head or motor domain contains both an actin-binding site and site of ATPase activity, a neck region with one to six IQ motifs that bind light chains or calmodulin, and a variable tail domain that binds cargos. In the inner ear, they have specialized functions associated with the stereocilia.

Myosin VIIa was the first protein in this family to be linked to deafness and is associated with both syndromic Usher syndrome type 1B (USH1B) and NSHL DFNB2 and DFNA11 (92, 162). One hundred mutations in myosin VIIa (MYO7A) have been found, with most linked to the syndromic form of deafness (Human Gene Mutation Database, **http://www.hgmd.cf.ac.uk/ac/index.php**). Myosin VIIa is expressed in the cytoplasm and stereocilia of both inner and outer hair cells of the cochlea (55). As mentioned previously, mutations in the *shaker1* (*sh1*) mouse mutant were discovered at the same time as the human *USH1A* mutations (48, 161). *Shaker1* mice are deaf and exhibit severe vestibular dysfunction, manifested as circling. The mechanism of this phenotype was revealed by detailed analysis of *shaker1* mice hair cells, which showed that they have raised thresholds for transduction currents and abnormal transduction channel gating (77). Moreover, the most recent data on myosin VIIa mouse mutants shows compelling evidence that this protein is directly involved in the control of actin dynamics within stereocilia, as it appears to regulate the organization of their height (**Figure 3*d***) (115). Although the hearing phenotype was readily apparent in the mice, the eye phenotype has been more difficult to decipher given that they do not demonstrate the retinal degeneration found in humans, although some alleles have reduced electroretinogram amplitudes (reviewed in Reference 170). A more thorough examination of some of the *shaker1* alleles demonstrated that the photoreceptor phenotype in the mice is manifested as increased opsin concentration in the photoreceptor connecting cilium, abnormal retinal pigment epithelium (RPE) melanosome localization, and motility and defective phagosome localization and digestion. From a biochemical point of view, some of the myosin VIIa mutations associated with USH1B abolish the actin-activated ATPase activity (159). The complexity of the Usher network is further emphasized by the interaction of myosin VIIa with a number of other proteins associated with Usher syndrome, including SANS (USH1G) and harmonin (USH1C), which further interacts with Usherin (USH2A) and VLGR1 (USH2C) (reviewed in Reference 76).

Myosin VI (MYO6), the second gene to be linked to deafness, was first found in the deaf Snell's waltzer mice in a positional cloning approach (7). It was only several years later that a human mutation was found. The *DFNA22* locus was originally represented by one Italian family, with a missense mutation in the head

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or motor domain of myosin VI (C442Y) (102). In recent years, additional mutations have been found in Belgian (59) and Danish (129) families. Recessive mutations in myosin VI have been found in Pakistani families (3). An essential component of all cells, myosin VI is involved in endocytosis, Golgi morphology, receptor clustering, cell migration, and vesicle transport (reviewed in Reference 140). Myosin VI is expressed in the cytoplasm of the hair cells, with increased levels in the cuticular plate and to some extent in the stereocilia (55, 56). Myosin VI is in a class of myosins that move toward the minus end of actin filaments, in the opposite direction that other characterized myosins move (164). In the inner ear, this means that myosin VI moves along actin toward the base of stereocilia. One of the more informative clues to myosin VI function has been through an ENU allele of myosin VI, Tailchaser (*Tlc*) (**Figure 3*e***) (56). A dominant missense mutation in the motor domain rendered the protein inactive. Transfection of myosin VI into epithelial cells and delivery of vesicles demonstrated that the *Tlc* mutant acts as a headless myosin. Furthermore, the ATPase rate for the mutant is lower, and it appears that the mutant form can no longer walk along actin, given that the head of the dimer remains stuck to actin. Indeed, myosin VI is concentrated in the tip of the stereocilia. The consequence is structural changes in the stereocilia, perhaps by dysregulation of actin treadmilling, that prevent the mice (and humans by extrapolation) from hearing. Indeed, a biochemical study of the human dominant missense mutation demonstrated that the mutation affects the ATPase activity and ADP dissociation rate of myosin VI, leading to an impaired processive movement of myosin VI (130). Identification of the cargos of myosin VI (such as Dab2, Sap97, and Gipc) is providing additional clues regarding myosin VI function (reviewed in Reference 140), but there is still crucial information missing regarding their role in hair cells.

A mutation in myosin IIIA(*MYO3A*) has thus far only been found in an extended Jewish family with Iraqi origin (154). Three different mutations in *MYO3A* have been found in DFNB30. This is an unusual form of deafness given that the mutations are recessive in nature, with late onset of HL. Class III myosins have a unique structure relative to the other myosins, as there is a kinase domain at its N-terminal end of the protein, predicted to regulate myosin IIIa motor kinetics (35). In the hair cells, myosin IIIa is expressed at the stereocilia tips, leading to speculation that it is involved in the mechanotransduction process (131). Myosin IIIa was recently reported to interact with espin, another deafness protein, and when coexpressed, they lead to stereocilia elongation and hence may work together to regulate stereocilia length (128).

Other myosins associated with HL include *MYO1A*, encoding a brush border myosin-I in the DFNA48 chromosomal region (33); *MYH9*, a nonmuscle heavy-chain gene associated with DFNA17 (82); and *MYH14*, a nonmuscle myosin heavy-chain gene responsible for DFNA4 (34).

Cell-Cell Junctions

The inner ear fluids' labyrinth is surrounded by a network of different epithelial cells, including the specialized sensory epithelium. Although some of these epithelial cells play a role in maintaining the homeostasis of the inner ear fluids, the entire epithelial network prevents leakage of ions and other essential elements outside these fluids. Hence, the occlusion of cell-cell interfaces is highly important to support and stabilize the inner ear structure. The contact between cells is established by a large number of cell-cell junctions, including gap junction, adherens junction, and tight junction protein complexes. The gap junction–forming proteins create a direct connection between the cytoplasm of two adjacent cells (reviewed in Reference 81). A representative group of connexin gap junctions is widely expressed in the inner ear and known to have a role in cell communication signaling and homeostasis. Compelling evidence for the importance of adherens junction proteins in inner ear function is nicely demonstrated by the cadherin transmembrane

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proteins (reviewed in Reference 136). Cadherins and protocadherins form cell-to-cell junctions. In the inner ear, they have a unique and specialized function as they form the tip links, the major site of mechanotransduction at the apical surface of the hair cell. Furthermore, cell junctions play another role in the auditory system, connecting cells to the extracellular matrix. The tight junctions regulate the paracellular movement of soluble molecules between cells and dictate epithelial transport properties (reviewed in Reference 142). In the inner ear, tight junctions contribute to the maintenance of the unique ionic composition.

In humans, several cell junction genes were shown to be involved in HHL. Mutations in two gap junction genes belong to the connexin family and are the major cause for NSHL, highlighted below. Mutations in the *OTOA* gene, encoding otoancorin, were shown to be associated with DFNB22, an autosomal recessive form of deafness (180). Otoancorin, predicted to be a glycosylphosphatidylinositol-anchored protein, was shown to be expressed in the greater epithelial ridge and in the spiral limbus, two attachment zones of the tectorial membrane. The collagen-rich tectorial membrane is a highly important extracellular matrix in the auditory system that resides on top of the sensory hair cells and participates in their mechanical triggering. The restricted expression pattern of otoancorin underlies the tectorial membrane attachment area, proposing that otoancorin functions as an anchoring protein mediating the attachment of the tectorial membrane. Other proteins involved in cell-cell junctions, but not discussed in detail in this review, include claudin 14 (DFNB29) (168) and tricellulin (DFNB49) (118), two tight junction proteins; and cadherin 23 (DFNB12) (12, 13) and protocaderin 15 (DFNB23) (4), two adherens junction proteins that were shown to form the tip links between stereocilia of the hair bundle (64).

Gap junction proteins. Connexins (Cx) are part of a family of gap junction proteins that reside in the cell membrane and create channels that connect the cytoplasm of two adjacent cells. Gap junctions can transfer both small and large components, enabling trafficking of ions, metabolites, and second messenger molecules that are essential for intracellular communication (reviewed in Reference 81). The assembly of gap junctions requires that each of the contacting cells will contribute one connexon, also known as a hemichannel. A single connexon is composed of six connexin transmembrane subunits, creating half of the channel. When two connexons of neighboring cells are docked together, an active gap junction is generated. Annotation of the human genome revealed 21 genes belonging to the connexin family (reviewed in Reference 169), whereas mutations in five of them, Cx26, Cx30, Cx31, Cx32, and Cx43, are involved in human deafness (Connexins-deafness, Homepage, **http://davinci.crg.es/deafness**). The heterogeneity of connexins involved in different human-inherited pathologies including the inner ear highlights their importance and function in cell-to-cell signaling and homeostasis of physiological processes (reviewed in References 47, 166). In this review, we concentrate on the most recent findings on Cx26 and Cx30, two prominent members of the connexin family in the inner ear.

The *GJB2* gene, encoding connexin 26 (Cx26), and the *GJB6* gene, encoding connexin 30 (Cx30), reside in close proximity to one another in the human genome and were mapped to the same *DFNB1* locus (29, 65). Mutations in both genes are the most abundant genetic alterations that lead to autosomal recessive nonsyndromic congenital HI in humans. In the inner ear, it was shown that Cx26 and Cx30 hemichannels coassemble to generate heteromeric gap junction channels (2, 40). Conditional disruption of Cx26 and null Cx30 cause mouse models to be profoundly deaf, exhibiting severe hair cell loss and supporting cell degeneration. Moreover, electrophysiological tests showed that these mice lack the essential endocochlear potential (24, 141), which was later related to the endothelial barrier of the stria vascularis capillaries in the Cx30 knockout mice (25). Interestingly, in both

mice, the unaffected gene retains the normal pattern of expression, forming homomeric gap junctions, but it cannot compensate for the loss of the other counterpart. Murine expression studies showed that Cx26 and Cx30 are colocalized and broadly expressed in several cell populations surrounding the scala media, including the supporting cells of the sensory epithelium, in the spiral ligament, and in the basilar portion of the stria vascularis (69, 84). The continuity of Cx26 and Cx30 expression all along the potassium recycling route, combined with the impaired endocochlear potential observed in the mouse models, led to the assumption that both connexins might have an important role in potassium propagation and homeostasis in the inner ear (175). Nevertheless, despite this suggestion, no direct evidence has yet been shown to support this hypothesis. Recent studies strongly suggest that Cx26 and Cx30 play a crucial role in cell-to-cell signaling in the auditory system. Electrophysiological patch clamp measurements on cochlear flat preparations from Cx30 null mice showed that the Cx26 homomeric channel retained conductance for ions but failed to propagate larger metabolites such as glucose to the cells, which can be transferred in the wild-type heteromeric channel. The reduction of glucose levels elevated the reactive oxygen species (ROS) in the cells, which eventually led to cell death (21, 22). In vitro studies with recombinant expression of Cx26 showed that several human mutations do not affect ion coupling but strongly inhibit biochemical coupling (10, 174). Additionally two companion articles showed that connexin deficiency dramatically decreases the calcium signal propagation in organotypic cochlear cultures. The cytosolic changes in the calcium levels affect the expression of NF-κB, a calcium-sensitive transcription factor that also controls connexin expression. Furthermore, the findings showed a coordinated regulation of Cx26 and Cx30 genes both on the transcriptional and functional level, which implicates intracellular calcium levels and activation of the NF-κB signaling pathway (6, 112). These findings highlight new functions of connexins in intracellular signaling pathways of the inner ear cells, providing mechanisms that explain the observed auditory pathology in humans.

Membrane Transport

The inner ear fluids have two pivotal roles that are essential for stimulating and activating the sensory hair cells. The first role is to absorb and mediate the mechanical forces of the sound waves from the conductive portions of the ear (the outer and middle ear) to the hair cells via fluctuation of the hair bundle. The second role is inherent in their unique ionic composition, which is crucial for the mechanoelectrical transduction of the hearing cascade.

Two extracellular fluids are encased in the cochlear duct: the endolymph in the scala media and the perilymph in the scala tympani and scala vestibuli (**Figure 1**). Both fluids are highly different in composition from other physiological fluids in the human body. The most prominent ionic characteristic of the endolymph is its high potassium and low sodium concentrations, compared with low potassium and high sodium concentrations in the perilymph. Upon deflection of the hair bundle stereocilia, mechanically gated ion channels are open, and an influx of primarily potassium, but also calcium and other cations from the endolymph, depolarizes the cells (reviewed in Reference 26). The efflux of potassium reaches the perilymph and is constantly recycled back into the endolymph through the spiral ligament and stria vascularis pathway in order to support the transduction of sound (reviewed in Reference 57). The maintenance of the inner ear fluids occupies a variety of cell types expressing a wide range of ion channels and transporters that need to work synergistically. Hence, a long list of human genetic mutations leads to HL as a result of dysfunction in one of the proteins that are responsible for inner ear homeostasis.

Transporters. The maintenance of the inner ear fluids' volume is no less important than their compositions. Mutations in the solute carrier gene *SLC26A4* encoding the pendrin protein

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lead to prelingual deafness diagnosed with hydrops of the inner ear cavities. In the cochlea, the increased volume can lead to expansion of the cochlear duct observed with Mondini dysplasia when the apical two cochlear turns are merged together. In the vestibule, the endolymphatic sac and duct are bulged, together with enlargement of the vestibular aqueducts (EVA) (116). Patient diagnosis, most often obtained by CT scan and MRI, demonstrates the abnormal characteristic of the inner ear and confirms the pathology (46, 114). Pendrin mutations in humans can lead to either autosomal recessive nonsyndromic deafness (*DFNB4*) or syndromic deafness known as PS, accompanied by enlargement of the thyroid gland or goiter. PS is considered to be the most common syndromic form of deafness (90). Pendrin is a transmembrane protein, functions as an anion transporter, and is expressed in several tissues in the human body including the thyroid, kidney, and inner ear (39, 75, 125). In the murine cochlea, pendrin is mostly expressed in the spiral prominence cells and actively transports bicarbonate ions into the endolymph. Pendrin knockout mice are profoundly deaf, exhibiting bulged endolymphatic spaces of the inner ear with striking similarity to the pathology observed in humans (38). Nevertheless, pendrin null mice do not develop thyroid clinical manifestations, and therefore the relation between pendrin mutations and syndromic thyroid pathology has yet to be determined. Electrophysiological measurements showed that pendrin disruption in mice leads to deafness via lack of an endocochler potential (EP), which subsequently was discovered to be due to the loss of the KCNJ10 potassium channel in the stria vascularis (137, 157). Extensive studies on the mouse model revealed that loss of pendrin bicarbonate secretion leads to acidification of the endolymph and inhibits the activity of pH-sensitive channels TRPV5 and TRPV6, resulting in an increase in calcium ion concentration (107, 158). Taken together, these results propose that pendrin is responsible for bicarbonate ion secretion into the endolymph, providing a buffering agent that supports the delicate equilibrium of the osmotic environment of the inner ear fluids and prevents dramatic changes in pH.

Ion channels. KCNQ (Kv7) is a small family of potassium channels, yet is highly important as four out of the five KCNQ members (KCNQ1 to KCNQ5) are known to be involved in different human diseases such as epilepsy, cardiac arrhythmia, and deafness (78, 110, 123, 155). All KCNQ proteins share similar structural properties containing six transmembrane domains. When four KCNQ subunits are assembled together, an active channel is generated. The fourth transmembrane domain in each subunit is characterized by positively charged residues that are believed to have a role in voltage sensing and accordingly affect the channel conformation and accessibility to potassium (reviewed in Reference 31). Hence, KCNQ proteins are classified as voltage-gated channels that depend on the membrane potential and can be activated upon depolarization of the cell membrane. The pivotal role of potassium in the inner ear fluids and its dynamics are emphasized by the fact that two members of the KCNQ family, KCNQ1 and KCNQ4, are essential for normal hearing.

Mutations in the *KCNQ1* gene in humans can lead to either inherited autosomal dominant long-QT (LQT) syndrome or the autosomal recessive Jervell and Lange-Nielsen (JLN) syndrome (110, 155). Both syndromes are characterized with cardiac arrhythmia, but the latter manifests congenital deafness as well. In the cochlea, KCNQ1 is associated with the KCNE1 peptide, and both are colocalized to the apical cell layer of the stria vascularis facing the endolymph (**Figure 2*f***). This valuable interaction changes the conductance properties of the channel, enabling the secretion of potassium into the endolymph (62, 122). Interestingly, it was shown that human mutations in the KCNE1 subunit can lead to JLN syndrome as well (133, 143), emphasizing that KCNQ1 cannot compensate for the absence of the KCNE1 subunit in the inner ear. Thus, disruption of either Kcnq1 or Kcne1 in the mouse leads to dysfunction of the channel and

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deafness with severe morphological abnormalities of the inner ear, including complete loss of sensory hair cells and stria vascularis atrophy (20, 85). In both mice, a drastic decrease of the endolymph fluid volume was observed, and collapse of Reisner's membrane on top of the sensory epithelium resembles the pathology observed, in humans (44). The availability of mouse models elucidated the function of the KCNQ1/KCNE1 channel in generating the electrochemical gradient of the endolymph and in maintaining its high potassium levels. The substantial roles of KCNQ1/KCNE1 channels in endolymph formation and homeostasis explain how mutations in either gene lead to severe congenital deafness in human.

Another member of the KCNQ family that was shown to be implicated in hearing impairment is the *KCNQ4* gene, which was mapped to the *DFNA2* locus (78). Human mutations in *KCNQ4* are known to cause slowly progressive HL inherited in an autosomal dominant mode. Expression studies in mice showed that KCNQ4 is expressed in the basolateral membrane of the outer hair cells and more prominently at the basilar region of the cochlea (**Figure 2*b***) (68). The gradient of KCNQ4 expression fits with the affected individuals' audiograms, indicating an earlier high-frequency tone loss, which correlates with the basilar region of the cochlea (27). Whereas KCNQ1 activity in the inner ear controls extracellular fluid homeostasis by secreting potassium into the endolymph, KCNQ4 activity is restricted to the intracellular electrical signaling pathway of the outer hair cells. The hair bundles of the outer hair cells are constantly immersed in potassium-rich endolymph, and upon deflection of the stereocilia, influx of potassium reaches the cytoplasm and depolarizes the cells. At this point, KCNQ4 channels dictate the efflux of potassium outside the cell in order to bring the cell back to the excitatory condition, ready to be activated by another sound. Disruption of the KCNQ4 channel in mice strikingly mimics the human hearing phenotype and reveals that the cause for the progressive HL is due to outer hair cell degeneration. Moreover, comprehensive electrophysiological measurements confirmed the hypothesis that the constant potassium overloading of the outer hair cells leads to cell death (67). Additional studies on mice postulating that the hearing impairment observed in humans cannot be explained only by outer hair cell dysfunction revealed that KCNQ4 is differentially expressed in the inner hair cells and in the spiral ganglion, suggesting an additional effect of KCNQ4 on the electrical signaling in the inner ear (9).

TF: transcription factors

Regulatory Elements

Cellular proliferation and differentiation, as well as structure and function, are controlled by regulated gene expression. Efficient and precise regulation of gene expression is implemented at the stage of transcription of DNA sequences into RNA by transcription factors (TFs). These factors are products of genes, whose specificity of transcription depends on binding of *trans*-acting factors to *cis*-binding regulatory elements (134). Several TFs harbor mutations leading to HL. Two are from the POU domain transcription factor family, with a bipartite DNA-binding domain that includes a homeodomain and POU domain. These include the DFN3 X-linked *POU3F4* gene (28). At least one mutation in the DFNA15 *POU4F3* gene may be associated with the loss of a nuclear localization signal (163). The EYA4 TF is crucial in a tightly controlled network of genes during inner ear development and is clearly relevant for later function of the organ of Corti, as mutations in this gene lead to progressive autosomal dominant HL DFNA10 (160). Finally, *TFCP2L3*, a mammalian homolog of the *Drosophila* gene *grainyhead*, is associated with mutations in DFNA28 HL (113). Most compelling, an association study in two independent populations placed this gene as a susceptibility gene for ARHL (146). Although we do not expand on TFs further in this review, other major types of newly discovered regulatory elements will be discussed in the next section.

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microRNAs

microRNAs (miRNAS), like TFs, are regulatory elements that alter gene expression (60). However, their mode of action is different than TFs. In mammals, cleavage by a number of RNase III endonucleases, including Dicer, generate miRNAs that eventually act as single-stranded molecules to pair with partially complementary sequences in the 3′ untranslated region of target mRNAs. The minimal miRNA sequence that is required for target identification is 6–8 nucleotides and is called the miRNA seed. They are relatively new to the scene of HHL. This may come as no surprise, given that, although the first miRNA was discovered in 1993 (87), their signficance only became apparent when a larger number was described in invertebrates and vertebrates (80, 83, 86). The most widely used database, miRBase (**http://microrna.sanger.ac.uk/sequences/**), lists no less than 9539 miRNAs representing 100 species in Release 13.0, as of March 2009 (51). *Homo sapiens* and *Mus musculus* have 706 and 547 miRNAs listed, respectively. The zebrafish (*Danio rerio*), another organism useful for inner ear research (reviewed in Reference 111), has 336 miRNAs.

miRNAs in the mammalian inner ear were first described in 2006 with the identification of miRNAs in sensory epithelium of mouse inner ears (165). Microarray analysis of the 344 miRNAs that were known at the time revealed that approximately one third are expressed in the inner ear. Most interesting, a triad found earlier to be expressed in the zebrafish inner ear hair cells (167) was detected specifically in mouse cochlear and vestibular hair cells, as well as by in situ hybridization. miR-183, miR-96, and miR-182 are clustered within 4 kb and transcribed in the order shown. This cluster maps to the critical region of DFNA50, a dominant progressive form of human HL. Most significant, sequencing of miR-96 in two DFNA50 families revealed mutations in the seed region (103). This is the first report of a miRNA mutation in a Mendelian disorder. As with other forms of human deafness, the mouse has been extremely informative about the effect of miRNA mutations on the inner ear. Fortuitously, another mutation in the seed region of the same miR was found at the same time in the ENU-generated *diminuendo* mouse mutant, which also develops a progressive HL (89). Microarray analysis of organ of Corti tissue, as well as bioinformatic-based Sylamer analysis, revealed altered expression of hundreds of genes in *diminuendo*. In particular, some proteins that are already known to be expressed in the sensory cells or associated with deafness themselves were downregulated, including oncomodulin, prestin, Pitpnm1, Gfi1, and Ptprq, although this is by far not a complete list of its targets. Clearly, the HLs in both the human and mouse forms of miR mutations are due to altered expression of many genes. Overall, loss of miRNAs in the inner ear by reducing Dicer, either at an early or late developmental stage, leads to HL in the mouse. A *Pax2-Cre;Dicer* conditional knockout was generated in which *Cre* expression, under the control of a *Pax2* promoter at early stages of inner ear development, in the otic placode, led to floxed deletion of *Dicer* in the inner ear sensory neurons as well as in the supporting cells and hair cells in all inner ear sensory epithelia. As a result, the inner ears developed abnormally, but hearing could not be examined given that the mice died during embryogenesis (138). Removal of *Dicer* later in development of the inner ear, through *Pou4f3-Cre* expression in hair cells, led to deafness in month-old mice, with loss of hair cell stereocilia as well as disorganization of hair bundles and stereocilia fusion (**Figure 3*f***), suggesting that miRNAs are crucial for hair cell survival (43).

Other Inner Ear Components

Given the complexity of the inner ear, exhibited in **Figures 1** and **2**, it should come as no surprise that there are many other genes associated with HL (Hereditary Hearing Loss Homepage, **http://webh01.ua.ac.be/hhh/**), whose proteins play a role in other functional entities of the ear.

Defects in the extracellular matrix of the inner ear are associated with HHL. Forms of both autosomal dominant and recessive DFNA8/DFNA12 and DFNB21 have been found with mutations in the *TECTA* gene, which encodes α-tectorin, a component of the tectorial membrane (149). Mutations in *COL11A2* affect the triple-helix domain of the collagen, type XI, alpha 2 protein, found in DFNA13 families (101). Mice deficient in this protein have moderate-to-severe HL associated with tectorial membrane abnormalities.

Other proteins include stereocilin, a hair bundle protein. Mutations in the gene *STRC* lead to DFNB16 HL (150). A study of stereocilin null mice revealed the nature of the origin of cochlear waveform distortions, which are essential for speech intelligibility (151). The *DFNA5* locus contains a gene, *DFNA5*, with mutations leading to HL; the gene was given the same name as the locus since for many years its protein identity or function was not known. Now, it appears to encode an apoptosis-inducing protein (145; K. Op de Beeck, G. Van Camp, S. Thys, N. Cools, I. Callebaut, unpublished results).

Whereas the majority of the known deafness genes are implicated with cochlear pathologies, *DFNB59* is the first reported gene that leads to deafness via neuronal dysfunction along the auditory cascade. Mutations in the *DFNB59* gene encoding the pejvakin protein are associated with autosomal recessive auditory neuropathy with bilateral prelingual HI (30). Interestingly, multiple sequence alignment analysis shows that pejvakin is a paralog of DFNA5. Expression studies in the mouse inner ear show that pejvakin is expressed all along the afferent auditory pathway. Pejvakin disruption in mice leads to deafness with impaired neural transmission, mimicking the observed pathology in humans.

Heterozygote mutations in the Wolfram syndrome 1 gene, *WFS1*, are associated with progressive hearing loss DFNA6/DFNA14 (11, 173). Homozygote mutations in the *WFS1* gene lead to Wolfram syndrome, an autosomal recessive disorder with diabetes mellitus, optic atrophy, and sometimes, but not always, deafness. The involvement of COCH, originally found in a human embryonic inner ear cDNA library, in human deafness was discovered in a unique fashion (121). Coch expression was detected in temporal bones derived from DFNA9 patients, and subsequent examination of the DNA from these patients led to the discovery of several mutations.

TMC1 mutations were found in both recessive (DFNB7/DFNB11) and dominant (DFNA36) forms of human HL (79). The discovery of the human genes led to the identification of the *Tmc1* mutations in the mouse, where the pathological consequences of *Tmc1* mutations could be studied (152). CRYM, an NADP-regulated thyroid-binding protein, may be involved in potassium-ion recycling (1). Given that the potential connection of CRYM in the inner ear was initially detected through cDNA microarray analysis and only then screened for mutations in HL, this gene is not found in families assigned a DFN number. Otoferlin (*OTOF*) mutations underlie DFNB9 prelingual deafness (171). Once again, the mouse was instrumental in revealing a significant function in the inner ear. A study of *Otof*-deficient mice revealed that the protein this gene encodes is essential for synaptic vesicle exocytosis at the auditory ribbon synapse (124).

The hurry-scurry (*hscy*) mouse model is a good example of how mouse genetics can contribute to the identification of new human deafness genes (93). A positional cloning approach revealed that a missense mutation in the *Tmhs* gene (tetraspan membrane protein of hair cell stereocilia) leads to deafness in hurry-scurry mice. The human *TMHS* gene, also known as *LHFPL5* (lipoma HMGIC fusion partner-like 5), was mapped to the *DFNB66/67* locus on chromosome 6 in the region homologous to mouse chromosome 17. Subsequent sequencing of the gene revealed mutations in *TMHS* in several families with recessively inherited NSHL (For example, see Reference 63).

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THE FUTURE OF RESEARCH IN THE GENETICS OF HL

Research in the genetics of HL has provided tremendous progress in our ability to diagnose mutations in HHL. Although most centers only screen for mutations in *GJB2* and *GJB3*, indications are often present for recommendation of screening particular genes. The question often arises: Which of the 46 known genes should be screened for? (58) For example, the presence of EVA is an indication for screening for *SLC26A4* mutations (116). Identifying mutations in specific ethnic groups, or categories of types of HL, such as association with vestibular dysfunction (121), can define which genes should be checked. Indeed, there are centers around the world that have a battery of tests available. Now more than ever, otolaryngologists and pediatricians should be aware of genetic testing algorithms for avoiding costly medical exams and replace them with relatively easy genetic tests in order to discover the etiology of HL in an individual (16). Knowing the mutation can allow the physician to make predictions regarding progression of HL or other abnormalities. However, more efficient methods of genetic testing are required in order to screen for all known genetic mutations simultaneously. The newly developed OtoChip, which is based on the Affymetrix CustomSeq GeneChips, sequences ~70,000 bases across 19 genes for HL, Usher syndrome, and PS (H. Rehm, personal communication). The OtoChip Test was launched into clinical use in June 2009 (**http://pcpgm.partners.org/LMM**). New sequencing technologies will undoubtably be applied to enhance diagnostic capabilities as well (96).

Beyond the monogenic forms of HL, many of which have been elucidated and described above, there are many other forms of hearing disorders with a genetic etiology. Complex forms of HL, including ARHL and NIHL, are due to both genetic and environmental factors. The first association studies for NIHL are currently underway, yielding promising results with the integration of high-throughput genotyping methods (reviewed in Reference 73). These include genes that protect against oxidative stress, and ion channels such as KCNQ1 and KCNE1. DNA variants that may influence susceptibility to ARHL are being discovered as well, including a gene already implicated in NSHL, *TFCP2L3* (113, 146).

One of the greatest challenges in inner ear research is to discover strategies to save our dying hair cells. Although we are born with approximately 15,000 hair cells, once they die, there is no chance to rescue them. Several strategies are being employed in an attempt to save or create new hair cells in the inner ear. Work so far has been performed in animal models, including guinea pigs and mice. Inner ear gene therapy has been conducted by retroviral insertion of the *Atoh1* gene into the cochlea of deafened guinea pigs (61) and in utero *Atoh1* gene transfer to produce functional supernumerary hair cells in the mouse cochlea (53). Cell replacement therapy is being designed based on inner ear stem cells that can differentiate to hair cells and neurons (98). Further studies in the genetics of HL will undoubtedly contribute to enhanced diagnostics and development of therapeutics, serving the HI community that supports and contributes to research in this area.

SUMMARY POINTS

1. The inner ear is an exquisite sensory organ that requires a myriad of genes and regulatory elements for its proper function. The significant worldwide efforts to clone these genes has yielded more then 130 mapped loci and 46 cloned genes, with still more to be found.
2. Knowing the identity of deafness genes is a first step in a long-distance run for understanding complex biological processes of the auditory and vestibular systems. Studies are currently being conducted to delineate the function of the genes and the proteins they encode as part of complex regulatory networks.

3. As demonstrated in this review, the genes known to be involved in human deafness encode proteins that are categorized under a wide spectrum of biological functions.
4. The striking similarity between the human and mouse inner ear places the mouse model at the frontier of research studies, enabling scientists to decode and analyze human pathologies. The availability of state-of-the-art technologies facilitates the generation of mouse models for each genetic alteration underlying human HL.
5. The genetics of the HL field of research has made it feasible to identify mutations by genetic screening, and in the future, screening can be used to identify individuals at high-risk for NIHL so that they can alter lifestyles to avoid frequent exposure to high-intensity sounds.
6. In the long run, the current comprehensive genetic and cell biological studies will establish a concrete basis for pioneering diagnostic and therapeutic approaches for HL, which will offer treatment for HI individuals as well as for preventive treatment for high-risk populations in order to avoid HL before it begins.

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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