Nonsyndromic Deafness - Molecular Update

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Abstract: In most cases, hearing loss is a disorder caused by both genetic and environmental factors. The molecular description of deafness has experienced remarkable progress in the last decade, and it is emerging from the use of contemporary methods of cell and molecular biology. Currently, through the application of clinical and molecular genetics it is possible to identify genes associated with inherited, nonsyndromic deafness, and balance dysfunctions of the human cochlea. This brief review provides insights into nonsyndromic hearing loss, since the identification of the molecular basis for the inner ear function provides the basis for developing rational new approaches to diagnosis, management and treatment of auditory and vestibular disorders.

Keywords: Nonsyndromic, hearing loss, sensorineural, molecular genetics, gene.

INTRODUCTION

Approximately half the cases of prelingual hearing loss are caused by genetic factors. Identification of deafness of genetic origin is a crucial first step to understand the function of genes in the auditory system. About 60% of the cases have hereditary etiology, 30% of the cases are acquired and, 10% are idiopathic. Nonsyndromic forms are responsible for 70% of the cases of hereditary etiology and syndromic cases represent 30% of them. Among the forms of inheritance, autosomal recessive is the most frequent one (75%-85%), followed by dominant inheritance (12%-13%) and X-linked or mitochondrial, with 2%-3% of the cases of nonsyndromic hearing loss [1].

The aim of the present review is to assess the most recent insights into described mutant genes that cause varied forms of nonsyndromic hearing loss: autosomal recessive, dominant, X-linked and mitochondrial. Selected articles are from OMIM® (Online Mendelian Inheritance in Man) [2] databases using MedLine, and the search mechanism used key words such as *"nonsyndromic", "hearing loss"*, *"sensorineural", "molecular genetics", and "gene"*. Selected articles were those that provided the most recent information about the genes involved and their respective proteins, their sites of expression in the cochlea and audiological clinical aspects. The symbols which used for the genes were approved by the HUGO Gene Nomenclature Committe (HGNC 2008) [3]. The selected references are in agreement with description of the genes from the Hereditary Hearing Loss Homepage [4] and OMIM [2] databases.

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NOMENCLATURE OF NONSYNDROMIC HEARING LOSS

Different chromosome loci of nonsyndromic forms of genetic deafness are named under the acronym DFN (from the word *deafness*) followed by the letters A or B, meaning autosomal dominant inheritance (DFNA) and recessive inheritance (DFNB), respectively. When using DFN isolated, it is X-linked deafness [5].

HEARING PHYSIOLOGY

The inner ear is a complex structure. A molecular description of its architecture is emerging from the use of contemporary methods of cell and molecular biology. With the application of clinical and molecular genetics, is now possible to identify genes associated with inherited, nonsyndromic deafness and balance dysfunction in the human cochlea. Therefore, to understand the consequences of gene mutations that regulate the hearing process one brief revision is necessary on the normal cochlear physiology. After the sound stimulus, the mechanical energy is converted into an electrical signal (mechanical-electrical transduction) in the outer hair cells of the cochlea. On the apical surface of these outer hair cells, there are specialized stereocilia which deflects in response to sound, which is secondary to the oval window stapes movement, moving the fluid that surrounds these hair cells. The deflection of stereocilia opens the transduction channels in them, allowing inflow of potassium from the endolymph to inner and outer hair cells of the cochlea causing depolarization of cell membrane and activating channels on the basolateral surface of the membrane, which are sensitive to voltage modifications. The subsequent influx of calcium causes release of neurotransmitters from the synaptic vesicles on to the primary afferent nerve ending synapses. Thus, after a sound stimulus, the inner and outer ear hair cells are hyperpolarized with high concentration of

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extracellular potassium. In order to make possible the next excitation, the potassium has to be removed. This movement of potassium ions from the ear hair cells to the cochlear supporting cells, going back to the endolymph, is made by intercellular specialized communications, the so-called gap junctions that exist among supporting cells, fibrocytes of the spiral ligament and the spiral limbus [6, 7].

MOLECULAR GENETIC OF NONSYNDROMIC HEARING LOSS

-Genetically Encoded Proteins with Expression in Cochlear Hair Cells (Table 1)

1. Unconventional Myosins

Myosins are actin-dependent motor proteins with ATPase activity and are categorized into conventional myosins (class II) and unconventional myosins (classes I and III through XV) based on their variable C-terminal cargo-binding domains. They are smaller than muscle myosin and for this reason they are called mini-myosins. Unconventional myosins act in intracellular movements. Their highly divergent tails are presumed to bind to membranous compartments, which are moved relative to actin filaments. In the inner ear, the actin filaments maintain the rigidity of stereocilia during the dynamic movements of the bundle [8, 9]. The different types of unconventional myosins related to the auditory system are the following:

a) Myosin IA

The unconventional myosin protein IA is encoded by the *MYO1A* gene, located on 12q13-q15. Mutations is this gene cause DFNA48, characterized by sensorineural bilateral hearing loss of variable degree, usually ranging from moderate to severe but never profound hearing loss. The hearing loss appeared to be caused by a completely penetrant mutation with a variable age of onset: age 30 to 35 years for the first symptoms, and age 20 to 25 years for the first audiometric abnormalities [10].

Table 1. Molecular Genetics of Genes Causing Hearing Loss with Expression in Cochlear Hair Cells

Gene Symbol	Chromosomal Locus	Protein	Function	Locus Name	Clinical Features/Age Onset
MYO1A	12q13-q15	myosin protein IA	unconventional myosins act in intracellular movements	DFNA48	hearing loss of variable degree/30 to 35 years
MYO3A	10p11.1	myosin protein IIIA		DFNB30	progressive deafness/2nd decade
MYO6	6q13	myosin protein VI		DFNA22 and DFNB37	progressive postlingual deafness/ childhood
MYO7A	11q.13.5	myosin protein VII		DFNB2 and DFNA11	moderate deafness/ 20 and 60 years
MYH9	22q12.2-q13.3	myosin protein IX		DFNA17	moderate-severe deafness/1st decade
MYH14	19q13	myosin protein XIV		DFNA4	progressive deafness/2nd decade
MYO15	17p11.2	myosin protein XV			profound congenital deafness
TMIE	3p14-p21	transmembrane inner ear	involved in protein and/or vesicle trafficking	DFNB6	severe-to-profound prelingual deafness
TMC1	9q13-q21	transmembrane cochlear 1	specific function is unknown	DFNB7/ DFNB11and DFNA36	profound prelingual deafness; progressive postlingual deafness/5 a 10 years
OTOF	2p22-p23	otoferlin	involved in vesicle membrane fusion	DFNB9	profound prelingual deafness
CDH23	10q21-q22	cadherin 23	involved in stereocilia organization and hair bundle formation	DFNB12	progressive prelingual deafness
STRC	15q21-q22	stereocilin	associated with the stereocilia	DFNB16	non-progressive deafness/childhood
USH1C	11p14-15.1	harmonin	part of a transmembrane complex that connects stereocilia into a bundle	DFNB18	severe prelingual deafness
PCDH15	10p11.2-q21	protocadherin-15	morphogenesis and cohesion of stereocilia bundles	DFNB23	prelingual deafness
RDX	11q23	radixin	linking actin to the plasma membrane	DFNB24	profound prelingual deafness
TRIOBP	22q13	trio- and f-actin-binding protein	controlling actin cytoskeleton organization, cell motility and cell growth	DFNB28	profound prelingual deafness

(Table 1) Contd…..

Gene Symbol	Chromosomal Locus	Protein	Function	Locus Name	Clinical Features/Age Onset
WHRN	9q32-q34	whirlin	coordinate polymerization of actin for the growth of the membrane in stereocilia	DFNB31	profound prelingual deafness
SLC26A5	7q22.1	prestin	essential for the auditory function	---	moderate-to-severe deafness
LHFPL5	6p21.3	lipoma h fusion partner protein	ear hair bundle morphogenesis	DFNB66/67	profound congenital deafness
DIAPH1 or *HDIA*	5q31	diaphanous	cell polarization and cytokinesis	DFNA1	Progressive postlingual deafness
KCNQ4	1p34	potassium channels	promote the outflow of potassium	DFNA2	progressive deafness/teens or 20 years
WFS1	4p16.3	wolframin	regulation of cellular Ca^{+2} homeostasis	DFNA6 (DFNA14/38)	low frequency deafness/2nd decade
POU4F3	5q31	transcription factor POU4F3	maturation and survival of the supporting cell layers	DFNA15	progressive deafness/18 and 30 years
ACTG1	17q25	actin-gamma 1	mediators of internal cell motility	DFNA20/26	progressive deafness/1st and 2nd decades
TFCP2L3	8q22	transcription factor CP2-like 3	transcription factor	DFNA28	progressive deafness/variable

b) Myosin IIIA

This unconventional protein is encoded by the *MYO3A* gene, located on 10p11.1. Expression of this gene is highly restricted, with the strongest expression in retina and cochlea (concentrated in inner and outer ear hair cells). This protein plays an important role in human hearing. Three different recessive mutations, with loss of function in the protein have been shown to cause bilateral progressive hearing loss (DFNB30), which affects primarily high frequencies, starting in the second decade, and at the age of 50, it reaches severe level in high and mid frequencies and moderate level in low frequencies [11].

c) Myosin VI

It is an actin-based molecular motor involved in intracellular vesicle and organelle transport. The myosin protein VI is encoded by the *MYO6* gene (6q13), and is concentrated in the cuticular plate in inner and outer ear hair cells. Mutations is this gene cause DFNA22 and DFNB37, characterized by progressive, and postlingual hearing loss with onset during childhood (8 to 10 years old to start symptoms, 6 to 8 years old for onset of audiometric alterations), and by the age of 50 approximately, affected individuals have profound sensorineural deafness [12,13].

d) Myosin VIIA

It is expressed in inner and outer ear hair cells and in a variety of ciliated epithelial cells, including retinal retinal photoreceptors. In the cochlea, the protein localizes along the stereocilia, the inner and outer hair cells, and supporting cells, as well as in the synaptic terminals. The myosin VIIA is encoded by the *MYO7A* gene, located on 11q.13.5. Mutations in this gene cause structural defects of protein and consequent alterations in the auditory function, responsible for two nonsyndromic forms of hearing loss, one with profound recessive autosomal inheritance - DFNB2, comprising different grades of vestibular dysfunction, and another one that is autosomal dominant - DFNA11, which is characterized by bilateral sensorineural hearing loss without vertigo or associated symptoms. There are symmetric gently sloping or flat audiograms with hearing loss at all frequencies. Most affected individuals between ages 20 and 60 have moderate hearing loss. When the mutations cause also retina cell abnormalities, the phenotypic sign is characterized as Usher syndrome. The chromosomal locus for one of the genetic types of Usher syndrome - USH1B was also mapped to the same region of chromosome 11, responsible for 75% of the cases of USH Type 1. Studies of mutation of the *MYO7A* gene causing DFNB2, DFNA11 and USH1B were the first to show that one gene could cause both non-syndromic and syndromic hearing loss respectively [14-16].

e) Myosin IXA

The conventional myosin protein IX is encoded by the *MYH9* gene, located on 22q12.2-q13.3. The membranous cochlea (especially, inner and outer ear hair cells, and spiral limbus, spiral ligament, and spiral ganglion) and saccule are affected, but the osseous labyrinth, the membranous utricle, and the semicircular canals are normal. The affected members have nonsyndromic hearing loss with an autosomal dominant mode of transmission (DFNA17). The hearing loss begins at the age of 10 and involves only the high frequencies; by the third decade of life, affected family members have moderate to severe deafness [17].

f) Myosin XIV

The myosin XIV is a member of the nonmuscle myosin II family of ATP-dependent molecular motors, which

interacts with cytoskeletal actin and regulates cytokinesis, cell motility, and cell polarity. The *MYH14* gene - myosin heavy chain 14, located on 19q13, encodes a member of the myosin superfamily, the myosin XIV, which is expressed as in ear hair cells as cochlear supporting cells up to the lateral wall cells, including Reissner's membrane. Mutations in this gene result in one form of hearing impairment (DFNA4), which is characterized by a fluctuating, inexorably progressive hearing loss, with onset in the second decade and leads from severe to profound impairment at the age of 40 [18].

g) Myosin XV

The myosin XV is encoded by the *MYO15* gene, located on 17p11.2. This myosin differs from others having a long N-terminal extension preceding the conserved motor domain. This protein is necessary for actin organization in the inner and outer hair cells of the cochlea, especially to the cuticular plate. Mutations in this gene have been associated with profound and congenital deafness due to an autosomal recessive mutation at a locus designated DFNB3 [19].

2. Transmembrane Inner Ear - TMIE

This protein, encoded by the *TMIE* gene (3p14-p21), may play some role in a cellular membrane location. It may reside within an internal membrane compartment and function in pathways such as those involved in protein and/or vesicle trafficking. Deafness (DFNB6) is caused by mutation in the transmembrane inner ear-expressed gene, characterized by severe-to-profound prelingual deafness [20].

3. Transmembrane Cochlear 1 - TMCI

The *TMC1* gene, located on 9q13-q21, is considered a member of a gene family predicted to encode transmembrane proteins, but its specific function is unknown; however, it is known to be required for normal function of cochlear hair cells. The protein Tmc1 is expressed in hair cells of the postnatal cochlea and vestibular end organs. Mutations in this gene have been associated with profound prelingual deafness (DFNB7/DFNB11) and progressive postlingual hearing loss (DFNA36), characterized by bilateral, symmetric hearing loss that begins from 5 to 10 years of age and rapidly progresses to profound deafness within 10 to 15 years. Affected individuals have no evidence of vestibular deficits in their developmental, and medical histories or upon physical examination [21].

4. Otoferlin

The otoferlin protein, which is expressed in inner ear hair cells, is involved in vesicle membrane fusion, and is encoded by the *OTOF* gene, located on 2p22-p23. Mutation in this gene causes DFNB9, characterized by prelingual profound hearing loss involving all frequencies, starting at birth or before the age of 2 years. None of the patients have balance problems. The audiometry shows no response at 100 dB at frequencies above 1,000 Hz in all affected subjects [22]. There is a specific type of deafness, caused by *OTOF* gene, termed 'nonsyndromic recessive auditory neuropathy' (NSRAN). Affected patients have hearing loss based on pure-tone audiometry and auditory brainstem response test results, which measure the overall auditory pathway, but have a normal otoacoustic emissions (OAE) test, which detects responses of the outer hair cells to environmental sound. Subjects with NSRAN can have varying degrees of hearing loss with poor speech reception out of proportion to the degree of hearing loss. Most subjects with NSRAN are not helped by hearing aids, but may be helped by cochlear implants [23].

5. Cadherin 23

The *CDH23* gene is a member of the cadherin superfamily encoding calcium dependent cell-cell adhesion glycoproteins. Expressed in the neurosensory epithelium, this protein is thought to be involved in stereocilia organization and hair bundle formation. This gene is located in a region containing the human deafness loci DFNB12 (10q21-q22) and USH1D (USH1D - 10q). The cadherin 23 protein is expressed in inner and outer cochlear hair cells, promoting strong adhesion between them, and in Reissner's membrane. Mutations of the *CDH23* gene cause DFNB12, characterized by moderate to profound high-frequency progressive sensorineural prelingual hearing loss. The average hearing loss is 84.0 dB. Vestibular function is normal [24].

6. Stereocilin

The stereocilin protein is expressed in the sensory ear hair cells and is associated with the stereocilia, is encoded by the *STRC* gene, located on 15q21-q22. Mutations in this gene cause DFNB16 characterized by bilateral, non-progressive, sensorineural hearing loss, with onset in early childhood. The hearing impairment involves all frequencies, is moderate in the range of 125-1000 Hz, but is severe in the higher frequencies. Vestibular function is normal, and there are no symptoms of tinnitus [25].

7. Harmonin

The *USH1C* gene, located on 11p14-15.1 encodes a protein, designate Harmonin, that contains the PDZ domain (Post synaptic density protein, Drosophila disc large tumor supressor, Zonnula occludens-1 protein). In the cochlea, harmonin is restricted to inner and outer hair cells, in which it is present in the cellular body and stereocilia and this protein is also expressed in the ribbon synapses. Defects in *USH1C* gene are the cause of DFNB18, characterized by prelingual and severe deafness. The functional characterization of the expression of harmonin protein provides the understanding of the pathogenesis of DFNB18 and USH1C syndrome [26,27].

8. Protocadherin 15

The *PCDH15* gene is also a member of the cadherin superfamily. The protocadherin-15 protein, which consists of a signal peptide, 11 extracellular calcium-binding domains, a transmembrane domain and a unique cytoplasmic domain, is encoded by the *PCDH15* gene, located on 10p11.2-q21. In the cochlea, protocadherin-15 is restricted to the cuticular plate of the inner and outer hair cells and external supporting

cells, beyond the spiral ganglion. Mutations in this gene have been associated with non-syndromic prelingual hearing loss (DFNB23), which is consistent with its location at the Usher syndrome type 1F (USH1F), critical region on chromosome 10 [28].

9. Radixin

Radixin is a cytoskeletal protein that may be important in linking actin to the plasma membrane. It is highly similar in sequence to both ezrin and moesin. The radixin, which is localizated along the length of cochlear hair cell stereocilia and in hair cells of the *crista ampullaris* at postnatal day 30, is encoded by the *RDX* gene, located on 11q23. The deafness (DFNB24) is characterized by prelingual onset and is bilateral and profound, and there is no vestibular dysfunction [29].

10. Trio- and F-Actin-Binding Protein - TRIOBP

The *TRIOBP* gene, located on 22q13, encodes a trio- and f-actin-binding protein that interacts with trio, which is involved with neural tissue development and controlling actin cytoskeleton organization, cell motility and cell growth. This trio-binding protein also associates with F-actin and stabilizes F-actin structures. This protein is expressed in sensory cells of the inner ear and colocalizes with F-actin along the length of the stereocilia. Mutations in this gene have been associated with a form of deafness (DFNB28), characterized by prelingual bilateral symmetrical and profound sensorineural hearing loss [30, 31].

11. Whirlin

This protein, encoded by the *WHRN* gene (9q32-q34), acts as an organizer of submembranous molecular complexes that control and coordinate polymerization of actin for the growth of the membrane in stereocilia of the inner and outer ear hair cells. The whirlin protein is similar to harmonin protein because it shares 95% of its three PDZ domains. Mutation in this gene is responsible for prelingual profound deafness (DFNB31) [32], and WHRN mutations may also cause USH2D syndrome [33].

12. Espin

The espin protein that is present in inner and outer ear hair cell stereocilia, is encoded by the *ESPN* gene, located on 1p36.1. A recessive mutation in this gene causes prelingual profound hearing loss (DFNB36), by ear hair cell degeneration, and the affected individuals show an independent ambulation delayed beyond 1.5 years of age by vestibular dysfunction [34].

13. Prestin

The *SLC26A5* gene, located on 7q22.1, is a member of the solute carrier (SLC) transporter family 26 (SLC26A). It encodes prestin, a motor membrane protein that is specifically expressed in outer hair cells of the cochlea and is essential for the auditory function. Intracellular anions are thought to act as extrinsic voltage sensors, which bind to this protein and trigger the conformational changes required for rapid length changes in outer ear hair cells. Mutations in this gene have been associated with nonsyndromic hearing loss, characterized by moderate-to-severe hearing loss and deterioration of frequency selectivity [35].

14. Lipoma H Fusion Partner Like Protein 5 - LHFPL5

The *LHFPL5* gene, located on 6p21.3, is a member of the lipoma HMGIC fusion partner (LHFP) gene family, which is a subset of the superfamily of tetraspan transmembrane protein encoding genes. This gene encodes lipoma h fusion partner protein, which is proposed to function in ear hair bundle morphogenesis. Mutations in the *LHFPL5* gene result in profound congenital deafness (DFNB66/67), and audiometric tests show loss of hearing greater than 70 dB for all frequencies tested [36-38].

15. Diaphanus

This protein belongs to a family of proteins related to formins, involved in cell polarization and cytokinesis. The diaphanous protein is encoded by the *DIAPH1* or *HDIA1* gene (5q31), that is homolog of the *Drosophila diaphanous* gene. In the cochlea, the protein is found in inner and outer hair cells and external supporting cells. Gene mutations affect the cytoskeleton of actin in outer ear hair cells and cause DFNA1, characterized by progressive low-frequency and postlingual hearing loss (with onset at about the age of 10, after language and speaking were learned). At the age of 40, approximately, hearing loss reaches severe level in all frequencies [39].

16. Potassium Channel, Voltage-Gated, KQT-Like Subfamily, Member 4 - KCNQ4

The protein subunit of family KCNQ of potassium channels, named KCNQ4 protein, is encoded by the *KCNQ4* gene, located on 1p34. In the cochlea, KCNQ4 channels are expressed not only in the outer hair cells, but also in the inner hair cells, and in the spiral ganglion, whose main function is to promote the outflow of potassium from these cells to the supporting cells [40]. Mutations in this gene are the cause of DFNA2, an autosomal dominant form of progressive hearing loss. The hearing loss first affect the high frequencies during the teens or 20s, becoming profound within 10 years [41,42].

17. Wolframin

This transmembrane protein participates in the regulation of cellular Ca^{+2} homeostasis, at least partialy, by modulating the filling state of the endoplasmic reticulum Ca^{+2} store. It is encoded by the *WFS1* gene (4p16.3), which is expressed in inner and outer ear hair cells and external supporting cells, beyond the spiral ligament, interdental cells, Reissner's membrane, and spiral ganglion. Mutations in this gene are associated with an autosomal recessive syndrome (Wolfram syndrome), and it can also cause DFNA6, also known as DFNA14 or DFNA38. Low frequency sensorineural hearing loss is an unusual type of hearing loss in which frequencies of 2000 Hz and below are predominantly affected. Many patients have tinnitus, but there are otherwise no associated features such as vertigo. Because high-frequency hearing is generally preserved, patients retain excellent understanding of speech, although presbycusis or noise exposure may cause

high-frequency loss later in life. DFNA6 worsens over time without progressing to profound deafness [43-45].

18. Pou Domain, Class 4, Transcription Factor 3 - POU4F3

The transcription factor POU4F3 protein is encoded by the *POU4F3* gene, located on 5q31. In inner and outer hair cells in the cochlea, the *POU4F3* gene seems to express during their migration from the supporting cell layers to the hair cell layer of the lumen, and also in their maturation and survival. A mutation found in *POU4F3* gene causes DFNA15, a progressive deafness, which starts between 18 and 30 years, and which reaches from moderate to severe level at the age of 50, approximately [46].

19. Actin-Gamma 1 - ACTG1

Actins are a family of highly conserved cytoskeletal proteins that are involved in various types of cell motility, and maintenance of the cytoskeleton. The alpha actins are found in muscle tissues and are a major constituent of the contractile apparatus. The beta and gamma actins co-exist in most cell types as components of the cytoskeleton, and as mediators of internal cell motility. Actin-gamma 1, encoded by the *ACTG1* gene (17q25), is a cytoplasmic actin found in nonmuscle cells, but much of the specialized ultrastructural organization of the cells in the cochlea is based on the actin cytoskeleton, especially inner and outer ear hair celss. Mutation in this gene is responsible for DFNA20/26, characterized by the onset in the first or second decade of life, high frequencies and progressive hearing loss involving all frequencies. Audiograms show a sloping configuration with age, resulting in profound hearing loss. The rate of progression is variable; a hearing aid wil be needed by the age of 20 [47,48].

20. Transcription Factor CP2-Like 3 - TFCP2

This protein, encoded by the ***TFCP2L3*** gene (8q22), is a member of a family of transcription factor genes whose archetype is TFCP2, a mammalian ortholog of the *Drosophila* gene 'grainyhead' (grh). In the cochlea, this protein is expressed in inner and outer hair cells, beyond external supporting cells, *stria vascularis*, Reissner's membrane, inner sulcus cells and interdental cells. Mutations in this gene have been associated with DFNA28, characterized by a mild to moderate deafness across most frequencies that progress to severe loss in the higher frequencies by the fifth decade, with variable age of onset [49].

- Genetically Encoded Proteins with Expression in Cochlear Non-Sensorial Cells (Table 2)

1. Connexin

Connexin protein is the structural component of intercellular gap junctions, which is responsible for the flow of potassium from the inner and outer ear hair cells, to the supporting cells, then the fibrocytes of the spiral ligament and spiral limbus, and back to the endolymph. One gap junction consists of a cluster of closely packed pairs of transmembrane channels, the connexons, through which materials of low molecular weight diffuse from one cell to a neighboring cell. A connexon is composed of a hexamer of connexins. The connexins are designated by their molecular mass. Another system of nomenclature divides gap junction proteins into 2 categories, alpha and beta, according to sequence similarities at the nucleotide and amino acid levels. For example, CX43 is designated alpha-1 gap junction protein, whereas CX32 and CX26 are called beta-1 and beta-2 gap junction proteins, respectively. This nomenclature emphasizes that CX32 and CX26 are more homologous to each other than either of them is to CX43 [50].

The different types of connexins related to the auditory system are the following:

a) Connexin 26

The connexin protein 26 is encoded by the *CX26* or *GJB2* gene, located on 13q11-12. Mutations in this gene are responsible for both forms of hearing loss - DFNA3 and DFNB1, characterized by prelingual, non-progressive, and profound deafness [51, 52].

b) Connexin 30

The connexin protein 30 is encoded by the *CX30* or *GJB6* gene, located at de same *CX26* gene locus-13q11-12. Mutations in this gene are responsible for both forms of hearing loss - DFNA3 and DFNB1 (both forms also are caused by *CX26* gene). If no mutation is found in *CX26* gene or in heterozygote patients for 35delG mutation, mutations of *CX30* gene, by its close relation (about 76% identical amino acids) and proximity of its chromosomic locus to *CX26* gene, they may be considered responsible for hearing loss, named similarly to *CX26* gene. This fact is explained, in addition to proximity, by the fact that *CX26* and *CX30* genes may form heterotopic channels of connexons, and they have the same cellular distribution in the cochlea. Therefore, pathophysiological hypotheses concerning hearing loss associated with both genes are similar [53,54].

c) Connexin 31

The connexin protein 31 is present in both spiral limbus and spiral ligament. The locus of *CX31* or *GJB3* gene (1p34) is the same for *KCNQ4* gene, which is expressed only in inner and outer ear hair cells, and if mutant it causes DFNA2. Owing to that, mutations in the *CX31* gene also cause dominant hearing loss, but although with expression in different site of *KCNQ4* gene, both received the same name - DFNA2 [55].

2. DFNA5

This protein is encoded by the *DFNA5* gene, located on 7p15. It is expressed in fetal cochlea, however, its function is not known. Mutations in *DFNA5* gene are the cause of DFNA5, characterized by progressive hearing loss starting in the high frequencies [56].

3. Pendrin/Solute Carrier Family 26, Member 4 - SLC26A4

The pendrin is encoded by the *SLC26A4* gene, located on 7q31. In the mature cochlea, the pendrin protein is expressed in spiral prominence, external sulcus cells, Claudius cells, Deiters' cells, and spiral ganglion Mutations in this gene are

Table 2. Molecular Genetics of Genes Causing Hearing Loss with Expression in Cochlear Non-Sensorial Cells

Gene Symbol	Chromosomal Locus	Protein	Function	Locus Name	Clinical Features/Age Onset
CX26 or *GJB2*	13q11-12	connexin 26	responsible for the flow of potassium	DFNA3 and DFNB1	non-progressive prelingual deafness
CX30 or *GJB6*	13q11-12	connexin 30		DFNA3 and DFNB1	non-progressive prelingual deafness
CX31 or *GJB3*	1p34	connexin 31		DFNA2	progressive deafness/ teens or 20 years
SLC26A4	7q31	pendrin	anion transporter	DFNB4	progressive congenital deafness
TMPRSS3	21q22	transmembrane protease serine 3	proteolytic activity	DFNB10/ DFNB8	congenital deafness/childhood
OTOA	16p12.2	otoancorin	noncollageneous glycoprotein unique to the inner ear	DFNB22	moderate-to-severe prelingual deafness
CLDN14	21q22	claudin 14	component of tight junction strands	DFNB29	profound congenital deafness
MARVELD2	5q12.3-q14.1	tricellulin	component of tricellular tight junctions	DFNB49	profound congenital deafness
PJVK	2q31.1-q31.3	pejvakin	essential in the activity of auditory pathway neurons	DFNB59	profound prelingual deafness
COCH	14q12-q13	cochlin	integrate into the extracellular matrix	DFNA9	progressive deafness/20 and 30 years
EYA4	6q22-23	eyes absent 4	continued function of the mature organ of Corti	DFNA10	progressive postlingual deafness
CCDC50	3q28-29	coiled-coil domain-containing protein 50	effector of epidermal growth factor-mediated cell signaling	DFNA44	progressive deafness/1st decade
CRYM	16p13.11-p12.3	mu-crystallin	possible involvement in the potassium-ion recycling system	DFNA-	progressive deafness/19 months
POU3F4	Xq21.1	pou domain, class 3, transcription factor 4	a role in potassium ion homeostasis has been postulated	DFN3	profound sensorineural deafness

also associated with Pendred syndrome (7q21-34), the most common form of syndromic deafness. DFNB4 is characterized by progressive hearing loss and widening of vestibular aqueduct, without thyroid affection [57].

4. Serine 3

The transmembrane protease serine 3 protein, that belongs to the serine protease family, is encoded by the *TMPRSS3* gene, located on 21q22. This gene was identified by its association with both congenital (DFNB10) and childhood onset deafness (DFNB8). It is expressed in fetal cochlea (supporting cells, scala vestibuli cells, and spiral ganglion) and many other tissues, and is thought to be involved or in the development and maintenance of the inner ear or in the contents of the perilymph and endolymph [58].

5. Otoancorin

All of the noncollageneous glycoproteins of the acellular gels of the inner ear that have been described in mammals, namely alpha-tectorin, beta-tectorin, and otogelin, are molecules unique to the inner ear. The otoancorin protein is encoded by the *OTOA* gene (16p12.2). Mutations in this gene have been associated with a moderate-to-severe prelingual deafness (DFNB22) [59].

6. Claudin 14

This protein, encoded by the *CLDN14* gene (21q22), is member of the claudin family, is an integral membrane protein and a component of tight junction strands. In the cochlea, this gene is expressed in tight junctions of inner sulcus cells, inner pillar cells, Deiters cells, and in inner and outer hair cells. Mutations in this gene are the cause of a profound autosomal recessive form of nonsyndromic sensorineural deafness (DFNB29) [60].

7. Marvel Domain-Containing Protein 2 - MARVELD2

This protein, encoded by the *MARVELD2* gene (5q12.3-q14.1), also named tricellulin, is an integral membrane protein concentrated at the vertically oriented tight junctions strands of tricellular contacts. In the inner ear, tricellulin is

concentrated at the tricellular tight junctions in cochlear and vestibular epithelia, including the structurally complex and extensive junctions between supporting and ear hair cells. Mutations in this gene are the cause of a congenital profound hearing loss (DFNB49) of all frequencies [61, 62].

8. Pejvakin

The pejvakin protein, which is essential in the activity of auditory pathway neurons, is encoded by the *PJVK* gene, located on 2q31.1-q31.3. Pejvakin is detected in the cell bodies of neurons of the afferent auditory pathway. Mutations in *PJVK* gene are the cause of deafness (DFNB59), characterized by prelingual profound deafness involving all frequencies, and with absent or severely abnormal auditory brainstem response but normal otoacoustic emissions (auditory neuropathy or auditory dyssynchrony) [63].

9. Cochlin

This protein, encoded by the *COCH* gene (14q12-q13), is detected in spindle-shaped cells located along nerve fibers between the auditory ganglion, and extracellular matrix, especially bone spiral lamina, spiral limbus and spiral ligament. These cells accompany neurites at the *habenula perforata*, the opening through which neurites extend to innervate ear hair cells. Mutations in this gene are responsible for DFNA9, which starts between the ages of 20 and 30 years, approximately. Initially, it is profound in high frequencies and shows variable progression to anacusis at the age 40-50. The spectrum of vestibular involvement varies from absence of symptoms to presence of vertigo and vestibular hypofunction. Histopathological analyses of temporal bone in patients with DFNA9 show deposits of mucopolysaccharides in the channels of the cochlear and vestibular nerves, which apparently, cause strangulation and degeneration of dendritic fibers [64].

10. Eyes Absent 4 - EYA4

The *EYA4* gene, located on 6q22-23, encodes a member of the eyes absent (EYA) family of proteins. This protein may act as a transcriptional activator and may be important for continued function of the mature organ of Corti. Mutations in this gene are associated with postlingual (onset in 2nd to 5th decades) progressive deafness (DFNA10). Losses start in mid frequencies and eventually involve low and high frequencies. There are no morphological anomalies of the cochlea, unlike those that result from mutations in the *EYA1* gene, which causes BOR syndrome (Branchio-Oto-Renal Syndrome [65].

11. Coiled-Coil Domain-Containing Protein 50 - CCDC50

This protein, encoded by the *CCDC50* gene (3q28-29), is associated with microtubules of the cytoskeleton and mitotic apparatus, which encodes an effector of epidermal growth factor-mediated cell signaling. Mutations in *CCDC50* gene are the cause of DFNA44. The hearing loss is initially moderate and affects mainly low to mid frequencies. Later, it progresses and involves all the frequencies and leads to a profound hearing loss by the 6th decade. The onset of the hearing loss occurs in the 1st decade of life (6-10 years of age). The DFNA44 hearing loss may result from a time-dependent disorganization of the microtubule-based cytoskeleton in the pillar cells and *stria vascularis* of the adult auditory system [66].

12. Crystallin, MU

There is strong expression of mu-crystallin protein, encoded by the *CRYM* gene (16p13.11-p12.3), only in the inner ear tissues - fibrocytes of spiral limbus and spiral ligament. Autosomal dominant hearing impairment (DFNA) has been identified at age of 19 months. Auditory brainstem responses, conditioned orientation reflex audiometry, and pure-tone audiometry examinations show bilateral moderate sensorineural hearing loss (average 50-60 dB) affecting all frequencies by a downsloping audiogram pattern. Hearing loss progresses from moderate to severe (70 dB) bilaterally at the age of 13 with the carolic test showing normal vestibular function [67].

13. Pou Domain, Class 3, Transcription Factor 4 - POU3F4

This protein, encoded by the *POU3F4* gene (Xq21.1), is expressed in the fibrocytes of the spiral ligament and bone spiral lamina, and in Reissner's membrane in cochlea. Mutations in this gene cause DFN3 (DFNX2), the first nonsyndromic X-linked deafness that was identified. This mixed type of deafness is characterized by both conductive hearing loss resulting from stapes fixation and progressive sensorineural deafness, and that sometimes a profound sensorineural deafness masks the conductive element. Computerized tomography demonstrated abnormal dilatation of the internal acoustic canal, as well as an abnormally wide communication between the internal acoustic canal and the inner ear compartment. As a result, there is an increased perilymphatic pressure that is thought to underlie the observed 'gusher' during the opening of the stapes footplate. Therefore, the DFN3 should be characterized, not by mixed conductive and sensorineural deafness associated with perilymphatic gusher at stapes surgery, but by profound sensorineural deafness, which is the *sine qua non* of this disorder, with or without a conductive component associated with a unique developmental abnormality of the ear [68].

- Genetically Encoded Proteins with Expression in Tectorial Membrane (Table 3)

The tectorial membrane is an extracellular matrix of the inner ear that contacts the stereocilia bundles of specialized sensory hair cells. Sound induces movement of these hair cells relative to the tectorial membrane, deflects the stereocilia, and leads to fluctuations in hair-cell membrane potential, transducing sound into electrical signals [69].

1. Collagen XI

The collagen 12 protein, one of the two alpha chains of type XI collagen, a minor fibrillar collagen, which is one of the components of the tectorial membrane, is encoded by the *COL11A2* gene, located on 6p21. Type XI collagen is a heterotrimer but the third alpha chain is a post-translationally modified alpha 1 type II chain. Mutations in this

Table 3. Molecular Genetics of Genes Causing Hearing Loss with Expression in Tectorial Membrane

Gene Symbol	Chromosomal Locus	Protein	Function	Locus Name	Clinical Features/Age Onset
COL11A2	6p21	collagen 12	one of the components of the tectorial membrane	DFNA13 and DFNB53	progressive deafness/2nd to 4thdecades profound prelingual deafness
TECTA	11q22	alpha-tectorin	non-collagenous component of the tectorial membrane	DFNA8/DFNA12 and DFNB21	non-progressive prelingual deafness

gene are associated with type III Stickler syndrome, otospondylomegaepiphyseal dysplasia (OSMED syndrome), Weissenbacher-Zweymuller syndrome, and DFNA13, which is characterized by progressive hearing loss beginning in the second to fourth decades, eventually making use of amplification mandatory [70,71], and DFNB53 which is characterized by prelingual, profound, nonprogressive, and nonsyndromic sensorineural hearing loss [72].

2. Alpha-Tectorine

The alpha-tectorin protein, one of the major non-collagenous components of the tectorial membrane, is encoded by the *TECTA* gene, located on 11q22. Mutations in the *TECTA* gene have been shown to be responsible for two forms of hearing impairment (DFNA8 and DFNA12) and a recessive form of deafness (DFNB21). The DFNA8 is characterized by moderate to severe, prelingual onset, and is relatively stable or non-progressive. Pure tone audiometry shows hearing loss between 60 and 80 dB, with a maximum at 2,000 Hz (severe range 1,000 to 6,000 kHz), and a U-shaped curve. The DFNA12 is characterized by mid frequency (500 to 2,000 Hz) hearing loss with prelingual onset. There is not progression with age. The DFNB21 is characterized by prelingual severe to profound sensorineural isolated form of deafness [73, 74].

- Nonsyndromic Hearing Loss Caused by Mitochondrial Mutations

Maternally inherited mitochondrial mutations give rise to nonsyndromic hearing impairment in some patients. However, for some mutations, patients have been found with additional symptoms accompanying the hearing impairment [75].

1. Ribosomal RNA, Mitochondrial, 12S - MTRNR1

The mitochondrial ribosome in the cochlea is the most likely target of aminoglycoside ototoxicity, since the 'natural target' of aminoglycosides is the evolutionarily related bacterial ribosome. In bacterial studies, regions of the small ribosomal RNA appear to be important in translational fidelity. Thus, the mitochondrial rRNA genes, and especially the *12S rRNA* gene (*MTRNR1*), were prime candidates for the site of the mtDNA mutations in maternally inherited aminoglycoside-induced deafness [76].

2. Transfer RNA, Mitochondrial, Serine, 1- MTTS1

To present, other described nonsyndromic mitochondrial mutations that cause hearing loss, followed or not by other symptoms, are located *tRNASer (UCN)* gene: a) palmo-plantar keratoderma with progressive, postlingual, and involved high frequencies, b) mitochondrial encephalopathy with cytochrome c oxidase deficiency associated with senso-rineural hearing loss, ataxia, myoclonic epilepsy, and mental retardation c) prelingual bilateral severe sensorineural deafness, and bilateral and permanent tinnitus with onset at age 20 years. There are no additional symptoms reported [77].

Recent studies suggested that mitochondrial mutation, such as the deletion mtDNAdel 4977 bp, may be responsible for familial cases of presbyacusis in some populations [78,79].

CONCLUDING REMARKS

The understanding of the complexities of the architectural organization necessary for cochlear and vestibular function has advanced so rapidly that the inner ear is becoming a major model for post-genomic studies, the attempt to discover for actual coding of the human genome genes. The identification of the molecular basis of inner ear function is setting the basis for developing rational new approaches to early diagnosis, management and treatment (gene therapy, drug treatment) of auditory and vestibular disorders.

CONFLICT OF INTEREST

There are no financial bounds or agreements between the authors and companies that may be interested in the material addressed in this Article.

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