Molecular Genetics Aspects of Hereditary Deafness

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Abstract
The genetic basis of hereditary deafness (HD) has undergone a dramatic transformation in the past 15 years. In the postgenomic era extensive research leading to the discovery of many genes essential for hearing was performed. Hereditary deafness is present in two forms – non-syndromic (70%) and syndromic (30%). Today more than 100 gene loci have been linked to nonsyndromic HD – dominant (DFNA), recessive (DFNB), X-linked forms (DFN) and some in the mtDNA. Over 60 genes are associated with syndromic HD like the most frequent syndromes of Waardenburg, Alport, Usher, and Pendred.

Taking in to account the exceptional genetic heterogeneity of HD it is not surprising that the genes found so far encode a large variety of proteins with different functions in the inner ear: connected to the structure and function of cochlear hair cells (7 types of myosin, otoferlin, cadherin, actin, stereocilin, harmonin, K and other ion channels, etc; proteins expressed in non-sensorial cells (connexins – 26, 30 and 31, pendrin, otoancorin, claudin14, etc.); proteins of tectorial membrane (collagen XI, alfa tectorine).The discovery of different type of gene mutations by linkage analysis, gene sequencing and whole exome sequencing with array analysis using OtoChip will enhance undoubtably the diagnostic capabilities, genetic counseling, screening and therapy of patients with HD in the future.

Keywords: hereditary deafness, nonsyndromic deafness, inner ear proteins, cochlea, hair cell

Introduction

Hearing loss is the most common birth defect. One of every 500 newborns has bilateral permanent sensorineural hearing loss ≥ 40 dB, and by adolescence the prevalence increases to 3.5 per 1000 (13)

The majority of the known genetic mutations affects the inner ear components and leads to sensorineural deafness. Non-syndromic forms (NSHD) are responsible for 70% of cases and the syndromic (SHD) cases represent 30% (16). More than 50% of prelingual deafness is genetic, most often recessive and nonsyndromic. In many world populations, 50% of persons with autosomal recessive nonsyndromic hearing loss have mutations in GJB2 The other 50% of cases are attributed to mutations in numerous other genes, many of which have been found to cause deafness in only one or two families (7, 28). A smaller number of genes are associated with middle ear conductive deafness.

Over 100 gene loci have been linked to dominant, recessive, and X-linked forms of non-syndromic deafness, and more than 60 genes have been identified, some of which are associated with both recessive and dominant deafness, as well as SHD. More than 1,000 discrete deafness-causing mutations have been described (Hereditary hearing Loss Homepage, http://hereditaryhearingloss.org/ (24, 25).

The aim of the present review is to assess the most recent insights into molecular aspects of the non-syn-

dromic hereditary deafness providing information about the genes, their respective proteins and sites of expression in the inner ear.

Non-syndromic deafness

Taking into account the extremely genetic heterogeneity of HD it is not surprising that the genes found so far encode a large variety of proteins with different functions in the inner ear – cytoskeleton components, extracellular matrix components, cell-cell junction, membrane transport, ion channels, fluid homeostasis, synaptic transmission etc. (Fig. 1). Mutations in their corresponding genes in nuclear and mitochondrial DNA are the genetic basis of different types of HD. The hereditary deafness (HD) is classified according to the pattern of inheritance: autosomal dominant (DFNA) with predominantly postlingual hearing impairment (some exceptions – DFNA3A, DFNA3B, DFNA6/14/38, DFNA8, and DFNA23) (Table 1); autosomal recessive (DFNB) with predominantly prelingual hearing impairment (exception – DFNB8 with onset at age 10–12 years but rapidly progressive, DFNB77 and DFNB91 (Table 2); X-linked (DFN) can be either pre- or postlingual; maternaly inherited gene mutations in mtDNA.

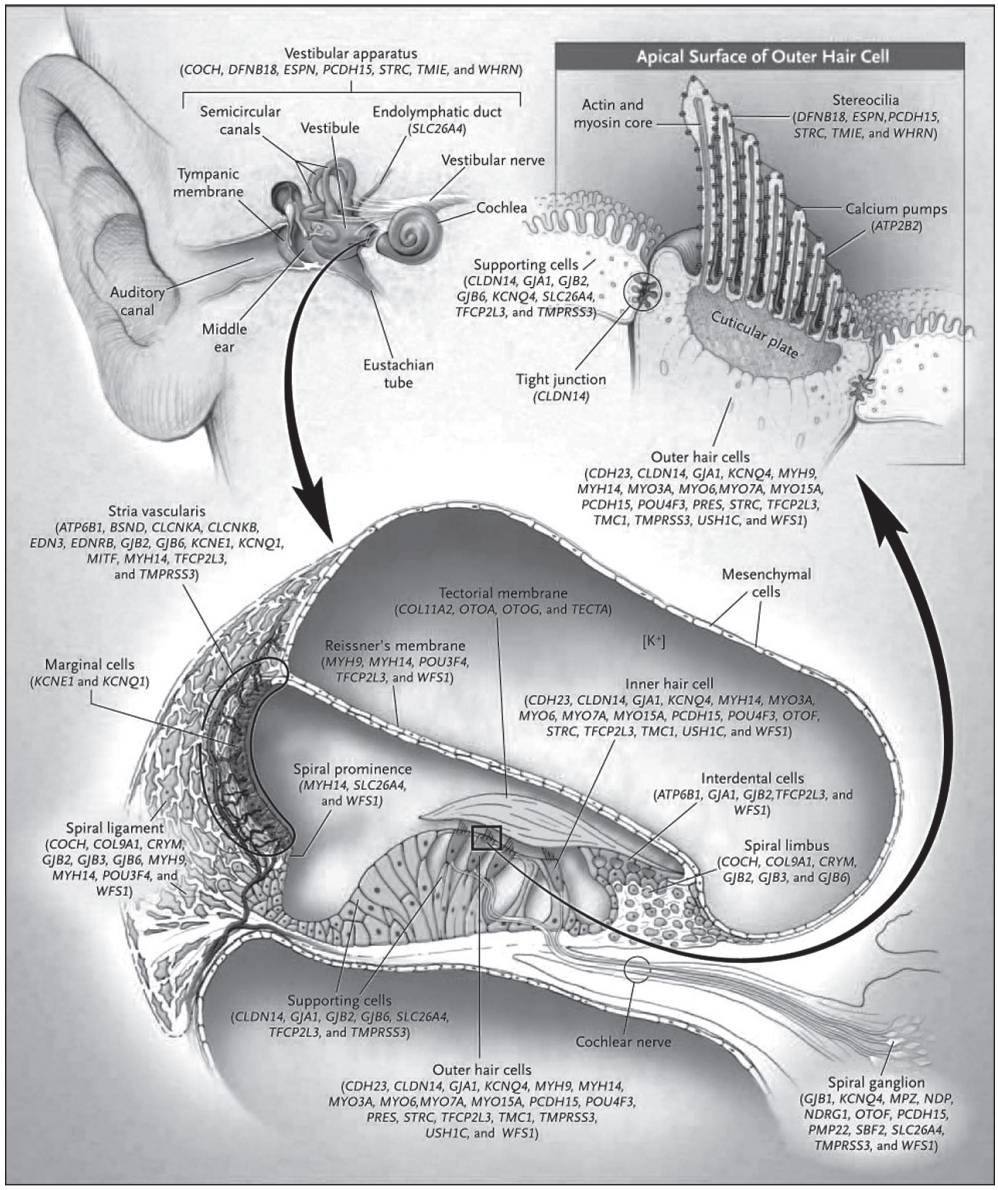

Figure1. View of the Outer, Middle,andInner Ear with a Cross-Sectional View of the Cochlear Duct and a View of Hair Cells.

The genes causing deafness and the locations of the products they encode are shown. The Hereditary Hearing Loss Homepage (www. webhost.ua.ac.be/hhh/) gives an interactive view of the expression of these genes.

Table 1. Autosomal dominant genes causing nonsyndromic hereditary deafness

Gene Locus	Gene Symbol	Protein	Clinical features:onset-decade/audioprofile
DFNA1	DIAPH1	Diaphanous	Postl. /1st/ Low frequency progressive
DFNA2A	KCNQ4	K+ channel	Postl. /2nd/ High frequency progressive
DFNA2B	GJB3	Connexin 31	Postl. /4th/ High frequency progressive
DFNA3A	GJB2	Connexin 26	Prel. / High frequency progressive
DFNA3B	GJB6	Connexin 30	Prel. / High frequency progressive
DFNA4	MYH14	Myosin 14	Postl. /2nd/ Flat-gently downsloping
DFNA5	DFNA5	DFNA5	Postl. /1st/ High frequency progressive
DFNA6/14/38	WFS1	Wolframin	Prel. / Low frequencies progressive
DFNA8/12	TECTA	α Tectorin	Prel. /Mid-frequency loss non-progressive
DFNA9	COCH	Cochlin	Postl. /2nd/ High frequency progressive
DFNA10	EYA4	Eyes absent4	Postl. /3rd, 4th/ Flat-gently downsloping
DFNA11	MYO7A	Myosin 7A	Postl. / 1st/ Flat-gently downsloping
DFNA13	COL11A2	Collagen11α2	Postl. /2nd/ Mid-frequency loss
DFNA15	POU4F3	Pou domen4F3	Postl. /2nd-6th/ High frequency progressive
DFNA17	MYH9	Myosin 9	Postl. /1st/ High frequency progressive
DFNA20/26	ACTG1	Actin γ1	Postl. /1st-2nd/ High frequency progressive
DFNA22	MYO6	Myosin 6	Postl. /1st/ High frequency progressive
DFNA23	SIX1	Six1	Prel. / Downsloping
DFNA25	SLC17A8	VGLUT3	Postl. /2nd-6th/ High frequency progressive
DFNA28	TFCP2L3	Tfcp2l3	Postl. /variable/ Flat-gently downsloping
DFNA36	TMC1	Tmc1	Postl. /1st/ Flat-gently downsloping
DFNA41	P2RX2	P2X2	Postl. /2nd/ Flat progressive
DFNA44	CCDC50	Ccdc 50	Postl. /1st/ Low to mild frequency progressive
DFNA48	MYO1A	Myosin1A	Postl. /3rd/ Progressive
DFNA50	MIRN96	miRNA96	Postl. /2nd/ Flat progressive gressive
DFNA51	TJP2	TJP2	Postl. /4nd/ High frequency progressive
DFNA56	TNC	TNC	Postl. /1st-2nd/ Progressive
DFNA64	SMAC/DIABLO	SMAC	Postl. /2nd-3rd/ Progressive

Prel. – prelingual; Postl. – postlingual; Wolframin – regulator of Ca^{2+} homeostasis; Six – DNA binding protein; VGLUT3 – vesicular transporter-3; Tfcp2l3 – transcription factor; Tmc1 – transmembrane hair-cell protein; P2X2 – receptor effector of EGF; Ccdc 50 – effector of epidermal growth factor(EGF); miRNA96 – microRNA96; TJP2 – tight junction protein; TNC extracellular matrix protein; SMAC – promotes apoptosis;

Table 2. Autosomal recessive genes causing nonsyndromic hereditary deafness

Gene Locus	Gene Symbol	Protein	Clinical features-onset/type
DFNB1A	CJB2	Connexin26	Prel. /profound/non-progressive
DFNB1B	GJB6	Connexin30	Prel. /profound/non-progressive
DFNB2	MYO7A	Myosin7a	Prel. /profound
DFNB3	MYO15A	Myosin15a	Prel. /severe to profound; stable
DFNB4	SLC26A4	Pendrin	Prel. /profound progressive
DFNB6	TMIE	Tmie	Prel. / severe to profound;stable
DFNB7/11	TMC1	Tmc1	Prel. / severe to profound;stable
DFNB8/10	TMPRSS3	Tmprss3	Postl.**or Prel. /progressive, stable
DFNB9	OTOF	Otoferlin	Prel. /severe to profound/non-progressive
DFNB12	CDH23	Otocadherin	Prel. severe to profound /non-progressive

DFNB15/72/95	GIPC3	Gipc3	Prel. /moderate-severe/non-progressive
DFNB16	STRC	Stereocilin	Prel. / severe to profoun/non-progressive
DFNB18	USH1C	Harmonin	Prel. / severe to profound /non-progressive
DFNB21	TECTA	αTectorin	Prel. / severe to profound /non-progressive
DFNB22	OTOA	Otoancorin	Prel. / severe to profound /non-progressive
DFNB23	PCDH15	Protocadherin	Prel. / severe to profound /non-progressive
DFNB24	RDX	Radixin	Prel. / severe to profound /non-progressive
DFNB25	GRXCR1	Grxcr1	Prel. /moderate to profound /progressive
DFNB28	TRIOBP	Triobp	Prel. / severe to profound /non-progressive
DFNB29	CLDH14	Claudin 14	Prel. / severe to profound /non-progressive
DFNB30	MYO3	Myosin3A	Prel. / severe to profound /non-progressive
DFNB31	WH	Whirlin	Prel. /profound /non-progressive
DFNB35	ESRRB	Esrrb	Prel. / severe to profound /non-progressive
DFNB36	ESPN	Espin	Prel. / profound/vestibular areflexia
DFNB37	MYO6	Myosin6	Prel. /profound /vertigo and retinitis pigmentosa
DFNB39	HGF	Hgf	Prel. / severe to profound ; downsloping
DFNB42	ILDR1	Ildr1	Prel. /profound/non-progressive
DFNB48	CIB2	CIB2	Prel.congenital /profound
DFNB49	MARVELD2	Tricellulin	Prel. / moderate to profound /stable
DFNB53	COL11A2	COL11A2	Prel. / severe to profound /non-progressive
DFNB59	PJVK	Pejvakin	Prel. / severe to profound /non-progressive
DFNB61	SLC26A5	Prestin	Prel. / severe to profound /non-progressive
DFNB63	LRTOMT	Lrtomt	Prel. / severe to profound /non-progressive
DFNB67	LHFPL5	LHFPL5	Prel. / severe to profound/non-progressive
DFNB70	PNPT1	PNPT1	Prel. /onset-early childhood
DFNB74	MSRB3	Msrb3	Prel. /severe to profound
DFNB77	LOXHD1	LOXHD1	Postl.1st decade /moderate to profound/ progressive
DFNB79	TPRN	Taperin	Prel. / severe to profound/non-progressive
DFNB84A	PTPRQ	Ptprq	Prel. /progressive/vestibular dysfunction
DFNB84B	OTOGL	Otogelin	Prel.congenital /moderate
DFNB86	TBC1D24	TBC1D24	Prel. /profound
DFNB88	ELMOD3	ELMOD3	Prel. /profound
DFNB89	KARS	KARS	Prel. /moderate
DFNB91	SERPINB6	SerpinB6	Postl.2nd decade /moderate/progressive
DFNB93	CABP2	Ca+bp2	Prel. /moderate to severe
DFNB98	TSPEAR	TSPEAR	Prel. /profound

*Prel. – prelingual,also includes congenital; Postl. – postlingual; **DFNB8 – caused by spicing mutation, onset-1st decade; LHFPL5 – Lipoma fusion partner protein; Ptprq -receptor; MSRB3 – methionin sufoxid reductase B3; HGF – hepatic grow factor; ESRRB – estrogen related receptor beta; CIB2 – CA+ and integrin-binding protein2; KARS – lysyl tRNA synthetase; CABP2 – Ca+ – binding protein 2; TSPEAR – thrombospondin-type laminin G domen;

Different forms of NSHD, responsible genes and encoded by them proteins in the inner ear will be presented below according to the recent literature data (5, 8, 16, 25)

1. Genes and cytoskeleton proteins expressed in cochlear hair cellsa). Unconventional (mini) myosins

Several myosins from the big myosins family (class I and III–XV) are present in the hair cells. They have specialized functions associated with the stereocilia, involved together with the actin filaments in the intracellular movements (5, 9).

Myosin VIIa was the first protein of this family, proved to be linked to deafness. This protein is directly involved in the control of actin dynamics within stereocilia and regulate their height. Different mutations in the gene MYO7A cause structural defects of the protein, responsible for two NSHD – one autosomal recessive – DNFB2 combined with

vestibular dysfunction, and another – autosomal dominant – DFNA11. As the protein is also present in the retinal cells some mutations cause SHD – subtype USH1B of Usher syndrome (5). This was the first gene proved to cause both non-syndromic and syndromic hearing loss. (Table l).

Myosin VI is an essential actin-based molecular motor, expressed in cuticular plate and stereocilia (5, 16). Mutations in the gene MYO6 determine two forms of progressive, postlingual, sensorineural HD – dominant (DFNA22) and recessive one (DFNB37) starting in the childhood.

Myosin IIIa has a unique structure, and is expressed at the stereocilia tips and is predicted to regulate motor kinetics. Recently it was reported that the protein probably interact with espin and other deafness proteins, and their co-expression regulate stereocilia length (20). Mutations of the gene MYO3A in some families lead to unusual form of HD – DFNB30, recessive with late onset. Mutated ESPN gene, encoding the protein espin cause prelingual, profound hearing loss with vestibular dysfunction (DFNB36) (14).

Other myosins associated with HD include: MYO1A, encoding a brush border myosin I, and DNFA48 deafness of variable degree; MYH9 coding for protein IX is associated with moderate-severe DFNA17 in the first decade; mutations in MYH14 result in DFNA4, progressive hearing loss with onset in the second decade;

b). actin-gamma-1 is a member of a family of highly conserved cytoskeleton proteins, involved in cell motility. The cytoplasmic gamma isoform of actin is a major component of outer and inner ear hair cells. The actin turnover including polymerization and depolymerization is dependent on a number of factors, like myosin motors and other proteins outlined below. Mutations of the gene ACTG1 are responsible for DFNA20/26, characterized by the onset in the first or second decade and progressive hearing loss involving all frequencies (29).

c). diaphanous – protein, related to the family of formins is expressed in the two types of hair cells and external supporting cells too. He is involved in the actin polymerization and cytokinesis. Alterations in the encoding gene DIAPH1 associate with DFNA1 which is postlingual, progressive, low-frequency in the beginning at age of 10 and later reachs severe levels in all frequencies.

d). other proteins of hair cells with important functions

– **stereocilin** is expressed in srereocilia of sensory hair cells, encoded by STRC which mutations cause, nonprogressive, sensoneural deafness (DFNB16), involving all frequencies with onset in early childhood.
– **espin** is an actin-bundling protein which is directly involved in stereociliary growth and length maintenance by stabilizing actin filaments. A recessive mutation in the gene ESPN causes profound prelingual deafness by hair cell degeneration and vestibular dysfunction (14).
– **whirlin, harmonin and radixin are** proteins expressed in hair cells that act as organizers of submembranous molecular complexes that control and coordinate polymerization of actin**.** Corresponding WHRN, USH1C and RDX mutations lead to autosomal recessive forms of prelingual, profound and severe deafness – DFNB31, DFNB18 and DFNB24. Some mutations in WHRN and USH1C genes may also cause subtypes of syndromic deafness in Usher syndrome patients (USH1C and USH2D).
– **trio-banding protein** is a specific actin-binding protein is controlling actin cytoskeleton organization, cell motility and growth. Mutations in the gene TRIOBP have been associated with profound deafness, DFNB28 (17).
– **protocadherin 15 and cadherin23** are adherents junction proteins, members of cadherin superfamily. They are involved in stereocilia organization (tip links between stereocilia), hair bundle and cuticular plate formation. The genes PCDH15 and CDH23 associate with DFNB12 and DFNB23, characterized as preligual, progressive, moderate to profound sensorineural hearing loss. Mutant alleles of these genes are known to cause subtypes USH1D and USH1F of Usher syndrome.
– **prestin** is member of the solute carrier transporter family 26, expressed specifically in outer hail cells. It is essential for the auditory function, acting as molecular motor that performs electro-mechanical conversion in outer cells, required for amplification of sound. Mutations in the respective gene, SLC26A5 lead to moderate-to severe deafness, DFNB61 (10).
– **otoferlin is** a specific protein located at the base of inner hair cells, next to synaptic region. It is involved in synaptic vesicle exocytosis at the

auditory ribbon synapse. It is encoded by OTOF gene, which mutations cause DFNB9, profound prelingual type (18).

– **wolframin** is a transmembrane protein expressed in outer and inner hair cells, and in external supporting cells, interdental cells and in the spiral ganglion too. It participates in the regulation of cellular Ca^{2+} homeostasis, modulating the filling state of the endoplasmic reticulum Ca^{2+} store. Heterozygote mutations in the encoding gene WFS1 are associated with unusual autosomal dominant type of low frequency sensorineural deafness (DFNA6/DFNA14/DFNA38) starting at second decade. Homozygote mutations lead to autosomal recessive syndrome of Wolfram, presented by diabetes mellitus, optic atrophy and sometimes deafness.
– **potassium channels proteins** are members of a small KCNQ family. These proteins are known as voltage-gate channels that depend on the membrane potential and can be activated upon depolarization of the cell membrane. Two of them, KCNQ1 and KCNQ4 are essential for normal hearing and are expressed in outer and inner hair cells and in the spiral ganglion too. The main function of the KCNQ4 is to promote the outflow of potassium from hair cells to supporting cells. Mutations in the KCNQ4 gene are the cause of slowly progressive deafness, DFNA2, starting with hearing loss first of high frequencies during the teens and becoming profound within 10 years (11). The gene is mapped in the same locus for connexin31 genes- GJB3. Mutations in the gene KCNQ1 are related to SHD in the autosomal recessive Jervell and Lange-Nielsen syndrome.

2. Genes and encoded proteins expressed in tectorial membrane

a). collagen 11A2 encoded by the gene COL11A2 is one of the components of the tectorial membrane. Mutations in this gene are associated with ultrastructural defects of the tectorial membrane and NSHD – DFNA13 and DFNB53. The first one is progressive with the onset between the 2nd-4th decades but the second one is profound, nonprogressive, prelingual. Some mutations in the gene lead to syndromic forms of deafness – Stickler syndrome, OSMED syndrome (otospondylomegaepiphyseal dysplasia).

b). alpha-tectorin is a major non-collagenous component of the tectorial membrane, and is encoded by TECTA gene (16). Mutations in this gene are responsible for two dominant forms of NSHD – DFNA8 and DFNA12 and one recessive – DFNB21. Several genotype – phenotype relationships have been defined. In DFNA8/12, the mutations in TECTA are missense mutations, with the audioprofile dependent on the location of the mutation. Missense mutations in zona pellucida(ZP) domain of the protein cause stable or progressive hearing loss involving the mid frequencies, while missense mutations in the zonadhesin (ZA) domain result in progressive hearing loss in the high frequencies.In DFNB21 the mutations in TECTA gene result in premature protein truncation and act like null alleles. Examples include frameshift mutations, nonsense mutations, and deletions. In all cases, the hearing loss is prelingual, symmetric, and moderate to severe in degree.

c). otoancorin is noncollageneous glycoprotein expressed in the greater epithelial ridge and in the spiral limbus. It acts as anchoring protein mediating the attachment of the tectorial membrane. Moderate to severe prelingual deafness (DFNB22) is caused by mutations in OTOA gene.

3. Genes and proteins expressed in cochlear non-sensorial cells

a). connexins (Cx) are part of a family of gap junction proteins, creating channels that connect the cytoplasm of two adjacent cells. Gap junctions transfer ions, metabolites, and second messenger molecules that are essential for intracellular communication (5). The gap junction consists of cluster of pairs connexons (hexamers of connexins). These transmembrane channels are responsible for the flow of K+ from the inner and outer hair cells to the supporting cells, than the fibroblasts of the spiral ligament and spiral limbus, and back to the endolymph. The main connexins related to auditory system are – Cx26, Cx30 and Cx31, encoded respectively by genes GJB2, GJB6 and GJB3. The genes GJB2 and GJB6 reside in close proximity to one other and were mapped to the same DFNB1 locus. Mutations in both genes are the most abundant genetic alteration

leading to autosomal recessive NSHD (Connexin-deafness homepage, http://davinci.crg.es/deafness/). Approximately 50% of the cases of autosomal recessive nonsyndromic hearing loss can be attributed to the DFNB1. The carrier rate in the general population for a recessive deafness-caused by GJB2 mutations is approximately one in 33, and more than 90 different mutations have been described in patients with NSHD. The most commonly found mutations of GJB2 in Caucasoid families especially in Mediterranean's and east Asian populations are deletions – 35delG and 235delC (28). Due to dominant-negative effect some mutations in these two genes can cause an autosomal dominant deafness (DFNA3) too.

The Cx 31 is present in both spiral limbus and spiral ligament. The corresponding gene GJB3 is responsible for progressive postlingual deafness, DFNA2. The same type of deafness is caused by another gene in the same locus-KCNQ4, encoding a protein of potassium channels.

b). proteins, components of tight junctions – claudin 14 and **tricellulin** are integral membrane proteins expressed in tight junctions of inner sulcus cells, inner pillar cells, Deiters cells, and inner and outer hair cells and make extensive junction between supporting and hair cells. Mutations in the corresponding genes, CLDN14 and MARVELD2 cause profound congenital deafness – DFNB29 and DFNB49 (16).

c). pendrin is a transmembrane protein, an anion transporter and is expressed in different tissues including thyroid, kidney, and inner ear. In the cochlea pendrin actively transport bicarbonate ions into the endolymph and is mostly expressed in the spiral prominence cells, Claudius cells, Deiters' cells and spiral ganglion. Mutations in the encoding gene SLC26A4 (Solute Carrier Family 26, Member 4) lead to progressive congenital deafness (DFNB4) and are also associated with Pendred syndrome, the second most common type of autosomal recessive syndromic hearing loss.

d). serine 3 – transmembrane protease with proteolytic activity, encoded by TMPRSS3 is expressed in fetal cochlea, mainly in supporting cells, scala vestibuli cells and spiral ganglion. It is supposed that the protein is involved in the development and maintenance of inner ear or in the contents of perilymph and endolymph. The gene mutations are responsible for two types of deafness, congenital DFNB10, and postlingual DFNB 8 with onset at age 10–12 years.

e). pejvakin is expressed in the cell bodies of neurons of the afferent auditory pathway. The encoding PJVK gene is the first reported to be a cause of deafness via neuronal dysfunction along the auditory cascade (3). The deafness, type DFNB59 is prelingual, profound and involving all frequencies.

f). cochlin is encoded by COCH gene and was found in spindle – shape cells located along the nerve fibers between the ganglion and extracellular matrix, especially bone spiral lamina, spiral limbus and spiral ligament. Different mutations in the gene lead to DFNA9, which is progressive, starting at age 20–30 years and accompanying with vestibular dysfunction. Deposits of mucopolysaccharides in the channels of cochlear and vestibular nerves that cause strangulation and dendritic fibers degeneration have been founded in temporal bones of patients (27).

4. Regulatory elements

a). transcription factors (TFs) are involved in the control of cell proliferation and differentiation by regulation of gene expression.

– the **POU3F4 TF** is found in fibroblasts of spiral ligament, bone spiral lamina, and in Reissner's membrane. Mutations in the corresponding gene cause the first nonsyndromic X-linked deafness DFN3 (4). The deafness is of mixed type, characterized by both conductive hearing loss from stapes fixation and progressive sensorineural deafness. Abnormal dilatation of the internal acoustic canal and increased perilymphatic pressure are found as a result of developmental ear abnormality.

– the **POU4F3 TF** is expressed in outer and inner hair cells during their migration from the supporting cells layer to the hair cell layer in the lumen and play an essential role in their maturation and survival. A mutation in the gene POU4F3 causes DFNA15, which is progressive and starts between 18 and 30 years of age.

– the **EYA4 TF** is an important component in a tightly controlled network of genes during the development of inner ear and clearly relevant for the function of the mature organ of Corti. Mutations in this gene cause postlingual pro-

gressive deafness with autosomal dominant pattern of transmission, DFNA10 (26).

– The **TFCP2L3 TF** has been found to be expressed in the hair, supporting and interdental cells, and also in the stria vascularis and Reissner's membrane. Mutations in the encoding gene associate with DFNA28, characterized by mild to moderate deafness across most frequencies and variable age of onset.

b). microRNAs (miRNAS) are small non-coding RNAs that like **TFs** regulate gene expression through the RNA interference (RNAi) and by inhibition of mRNA translation. More than 700 human miRNAs are known and by microarray analysis it was proven that approximately one third of them are expressed in the inner ear (http://microrna.sanger.ac.uk/sequences). The first report in 2009 revealed mutation in the gene MIRN96, encoding miR-96 in a family with progressive nonsyndromic deafness, DFNA50 (12)... Extensive transcriptomic and proteomic approaches have been undertaken on mouse models to identify miRNAs and their targets. The results suggest that miRNAs mutations play an important role in pathogenesis of deafness, altering expression of many genes. In the future it is possible miRNAs to be used in regeneration of inner ear hair cells and new therapeutic aapproaches (12, 19).

5. Mitochondrial nonsyndromic hearing loss and deafness

Most of mutations in mitochondrial DNA associate with a large spectrum of maternally inherited multisystem disorders. Although the majority of cases of HD are caused by nuclear gene defects, it has become clear that mutations in mtDNA can also cause hereditary deafness. Nonsyndromic mitochondrial hearing loss and deafness is caused mainly by mutations in either MT-RNR1 or MT-TS1 genes and is characterized like moderate-to-profound one. (1, 6, 8). Mutations in MT-RNR1 can be associated with predisposition to aminoglycoside ototoxicity and/or late-onset sensorineural hearing loss. Mutations in MT-TS1 are usually associated with childhood onset of sensorineural hearing loss. Hearing loss associated with aminoglycoside ototoxicity is bilateral and severe to profound, occurring within a few days to weeks after administration of any amount of an aminoglycoside antibiotic.

a). MT-RNR1 gene encodes the 12S rRNA. One point mutation in this gene, 1555A > G, is a frequent cause of maternally inherited nonsyndromic hearing loss. In some individuals with the this mutation, the hearing loss is induced by the administration of appropriate doses of aminoglycosides; however, phenotypic variation is great, consistent with the effect of modifier genes. The percentage of individuals with mitochondrial nonsyndromic hearing loss and deafness caused by the m.1555A > G varies by ethnicity(6, 8): approximately 17% in a cohort of European origin and in a Spanish cohort; 5% to 13% in persons of Chinese and east Asian background.

b). MT-TS1 gene encodes for the tRNAser. In two families with heteroplasmy for point mutation of this gene, transition 7445A > G, have been identified however, the penetrance of hearing loss was low, and it has been suggested that MT-TS1 mutations play an insignificant role in hearing loss. Fewer than 2.5% of individuals with mitochondrial nonsyndromic hearing loss and deafness caused by mutation of MT-TS1 have one of the following alleles: m7443A > G, m7444G > A and m7445A > C. Although hearing loss associated with mutations in MT-TS1 is considered nonsyndromic, the m.7445A > G substitution is also associated with palmoplantar keratoderma in some families.

c). MT-CO1 gene encodes cytochrome c oxidase subunit 1. Six individuals with severe-to-profound deafness showed cosegregation of a homoplasmic 7444G > A transition of MT-CO1 and the 1555A > G mutation in MT-RNR1 (15). Five of the six patients showed maternal inheritance and two had a previous history of aminoglycoside use. As opposed to the variable hearing loss associated with MT-RNR1 1555A > G, all individuals with this double mutation showed severe-to-profound impairment of hearing and the gene penetrance was complete.

Recent studies suggest that mtDNA mutations like deletions may be responsible for familial cases of presbyacusis in some populations (16).

Molecular genetic testing for deafness

Since the discovery of the first human gene, GJB2 encoding connexin 26 in 1997 by D. Kelsell and collaborators (24), a remarkable progress has been made in identifying deafness genes, and under-

standing mechanisms of hereditary hearing loss as well. After the completion of the human genome project in 2001 many deafness genes have been mapped and mutations for the majority of HD have been discovered by linkage analysis and direct DNA Sanger sequencing. These studies have had an immense impact in both the research and clinical practice They have led to the discovery of new non-syndromic hearing loss (NSHL) and syndromic HL genes that have been rapidly translated to the clinical arena.These genes were sequenced one by one after ranking them on the bases of hypothesis about gene function or expression. This approach was not optimal as it is both time and cost – consuming, and don't allow examination of a large number of genes simultaneously. So the extreme genetic heterogeneity and the frequent lack of phenotypic variability make genetic diagnosis of NSHD difficult using single-gene screening techniques.

For this reason genomic technologies like next-generation sequencing (NGS) also called massively parallel sequencing (MPS), and second-generation sequencing, has been developed over the past five years to meet this demand (22, 23, 24).

Several groups have developed multi-gene screening panels for NSHL. These screening panels vary by laboratory both in the techniques used and the number of genes sequenced. Some laboratories target only reported mutations in several genes, while other laboratories sequence all known genes responsible for NSHL. It is likely that as such tests become more widespread, the management of genetic hearing loss will change to a single comprehensive genetic test for all types of hearing loss.

There are three commercial MPS platforms routinely used in the research of deafness gene mutations:- HHL APEX, OtoChip and OtoScop (23). The first one use single nucleotide extension microarray technology and allows screening only of target specific known deafness mutations without direct sequencing. Resequencing microarraay is a basic technology of the second one, OtoChip, which examines 19 of deafness genes and is unable to detect gene insertion and deletions. The third one, OtoScop testing platform is most promising as allow screening of 66 deafness genes This platform is performed in two steps – fist capture all of the exons of the NSHD genes by SBTE (solution-based target enrichment followed by massively parallel sequencing.Using this new genomic technology four genes for SHD and four NSHD genes, like TPRN (DFNB79), GPSM2 (DFNB82), CEACAM16 (DFNA4), SMPX (DFNX4) have been recently identified (23).

In conclusion it is noteworthy that NGS has proven to be optimal genetic diagnostic technology to discover deafness mutations. Moreover, identifying all genes involved in the pathogenesis of deafness might facilitate a greater understanding of auditory complexity and mechanisms leading to deafness. It is setting the basis for rational new approaches to early diagnosis, genetic counseling, prenatal diagnosis and probably in the near future regeneration therapy.