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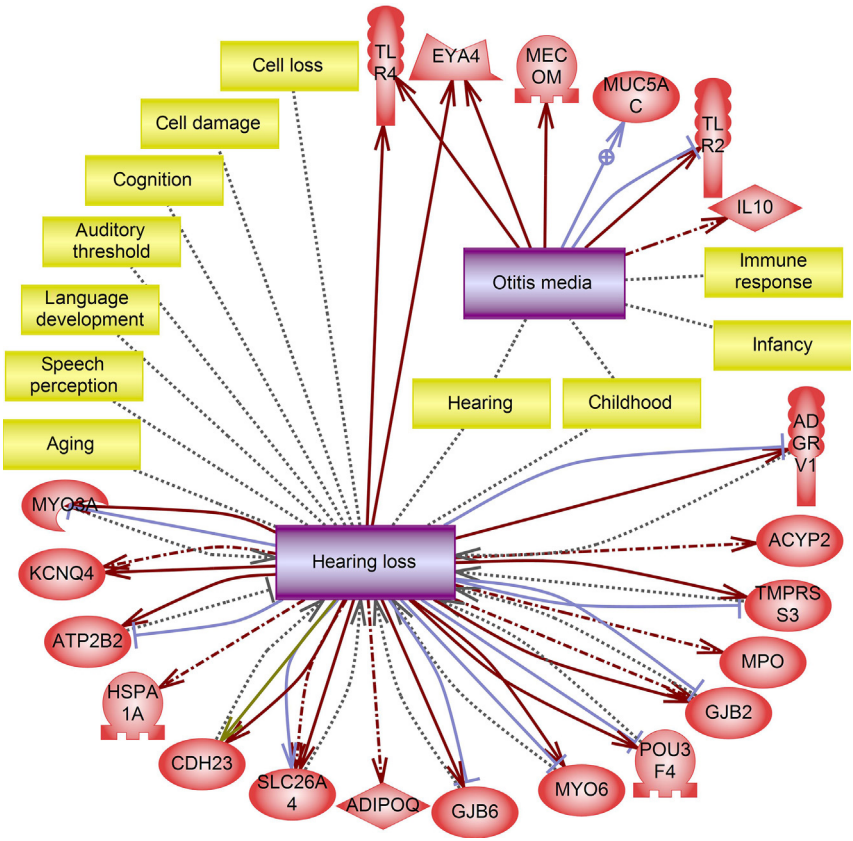
CHAPTER

7

Diseases of the ear



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OUTLINE

Diseases of the ear are as diverse as diseases of the eye and can affect both hearing and the sense of balance. Ear diseases can be classified by the part of the ear affected, namely, the outer, middle, or the inner ear.

In children with normal hearing, inflammatory disorders caused by infections of the middle ear (otitis media) are the most common ear illnesses. Inflammation of the middle ear often causes acute pain, and untreated otitis media may lead to severe complications such as perforation of the eardrum or even bacterial meningitis.

Many of older adults experience some level of hearing loss, either a partial loss or the total inability to hear (deafness).

Several factors can lead to either a partial loss or the total inability to hear (deafness) including exposure to noise, a hereditary predisposition, chronic infections, traumas, medications, and aging. In nonsyndromic hearing loss, there are any associations of loss of hearing with additional manifestations. In contrast, syndromic hearing loss occurs with signs and symptoms affecting other parts of the body. Hearing loss is often inherited with approximately 75%–80% of observed cases inherited in a recessive manner and 20%–25% with a dominant mode of inheritance.

CHAPTER

7.1

Hearing loss

Hearing loss is a complex condition. The nonsyndromic hearing loss is a partial or total loss of hearing not associated with other signs and symptoms. In contrast, syndromic hearing loss occurs with signs and symptoms affecting other parts of the body (Genetics Home Reference, https://ghr.nlm.nih.gov).

Nonsyndromic hearing loss is classified in several different ways, for example, by the pattern of inheritance (autosomal dominant, autosomal recessive, X-linked, or mitochondrial). The causes of nonsyndromic hearing loss are complex with mutations in more than 90 genes associated with nonsyndromic hearing loss to date. Many of these genes handle the development and function of the inner ear.

Age-related hearing loss (ARHL, also known as presbycusis) is a decrease in hearing ability that happens with age. ARHL develops from a combination of genetic, environmental, and lifestyle factors. Age-related hearing loss is most commonly related to dysfunctions in the inner ear, where sound waves turn into nervous impulses (Genetics Home Reference, https://ghr.nlm.nih.gov).

Mutations in genes encoding structural proteins specific for cochlear hair cell may cause hearing loss:

Pathway 1. *Dysfunction of cochlear hair cell stereocilia proteins in hearing loss* (Fig. 1).
Pathway 2. *Dysfunction of cochlear hair cell synapse proteins in hearing loss* (Fig. 2).
Impairment of mechanoelectrical transduction and potassium (K^+) cycling in the inner ear is the main reason for congenital hearing loss:
Pathway 3. *Deficiency of potassium cycling in hearing loss* (Fig. 3).

Key cellular contributors and processes

Cochlear hair cell
Cell

Cochlear hair cells are the sensory cells of the auditory system. These cells possess stereocilia connected to the tectorial membrane. During auditory stimulation, sound waves in the cochlea cause deflection of the hair cell stereocilia, which creates an electrical signal in the hair cell.

Cochlear
Anatomic structure

Cochlea is a snail-shaped canal in the osseous labyrinth of the inner ear, which contains the sensory organ of hearing—the organ of Corti.

Inner ear
Anatomic structure

The inner ear is the innermost portion of the ear that contains organs responsible for hearing and the sense of balance. Located in the temporal bone, the inner ear has three essential parts: cochlea, vestibule, and semicircular canals.

Mechanoelectrical transducer channel
Anatomic structure

The mechanoelectrical transducer (MET) channels are ion channels on the tips of stereocilia. Deflection of stereocilia provokes mechanical opening of these channels and the entrance of cations that generates action potential.

Organ of Corti
Anatomic structure

The organ of Corti is the auditory organ situated in the cochlea of the inner ear. The sensory hair cells that make up the organ of Corti are responsible for the transduction of the auditory impulse into neural signals.

Ribbon synapses
Cell

A ribbon synapse is a neuronal synapse structurally different from other synapses by the presence of an electron-dense structure called synaptic ribbon, which helps to keep synaptic vesicles near the active zone. Ribbon synapses are found in various sensory receptor cells, for example, auditory hair cells of the cochlea, and characterized by increased performance.

Stereocilia

Anatomic structure

Stereocilia are thin projections on the cochlear hair cells that respond to fluid motion and are involved in mechanosensing. Despite a similar name, stereocilia are different from cilia (microtubule cytoskeleton–based structures) and contain actin cytoskeleton, similarly to microvilli.

Tectorial membrane

Anatomic structure

The tectorial membrane is a band of extracellular matrix in the cochlea located above the inner and outer hair cells of the organ of Corti. The tectorial membrane is connected to stereocilia of the outer hair cells and participates in mechanotransduction. During auditory stimulation the tectorial membrane directly stimulates the outer hair cells and creates liquid movements that stimulate the inner hair cells.

Pathway 1

Dysfunction of cochlear hair cell stereocilia proteins in hearing loss (Fig. 1)

Incoming signals

The transduction of sound waves within the ear involves movement of parts of the cochlea in the inner ear including the tectorial membrane and the fluid within the labyrinth termed endolymph. Endolymph, found inside the cochlear duct (i.e., the scala media), is very rich in potassium (150 mM) and very poor in sodium (1 mM). These concentrations are unique among physiological fluids. Hearing depends on the high K^+ concentration in endolymph. Fluid motion and tectorial membrane vibrations bend protrusions of hair cell membranes (stereocilia). Stereocilia movements and K^+ and Ca^{2+} influx transform mechanical impulses (i.e., sound waves) into electrical impulses in the form of action potentials. Loss-of-function mutations in different genes that encode critical proteins in stereocilia of the cochlear hair cell impair mechanoelectrical transduction and therefore cause hearing loss. Congenital hearing loss is most often associated with dysfunction of actin-myosin complex organization within the ear. The pathway reconstructed here reviews all known mutations together although usually one mutated gene underlies inborn hearing loss.

Outcome effects

Bending of higher stereocilia under the influence of a sound wave causes mechanical opening of the mechanoelectrical transducer (MET) channels on the membranes of lower stereocilia by tensioning the tip of each lower stereocilium with the side wall of its associated higher one. K^+ and Ca^{2+} enter the stereocilium through MET channels and lead to the transformation of the mechanical impulse or sound wave into an electrical impulse or action potential. Dysfunctions in stereocilia proteins lead to the impairment of their movements, the inability of mechanoelectrical transducer channels to open, and the subsequent failure to transform a sound wave into an electric impulse.

Signaling

Stereocilia movement is an actin-/myosin-dependent process. The loss of function of a number of myosins (such as MYO3A, MYO6, MYO7A, MYO15A, MYO1A, MYO1C, MYO1F, MYH9, and MYH14) has been shown to be associated with both dominant and recessive forms of hearing

loss. *MYO7A* mutations, for example, may cause a rare disorder known as Usher syndrome type IB.

Dysfunction of several proteins controlling actin filaments in the cytoskeleton may be the reason for some subtypes of nonsyndromic hearing loss. Homer scaffolding protein 2 (HOMER2) regulates actin dynamics in stereocilia through its interaction with the cell division cycle 42 (CDC42) protein. Diaphanous-related formin 1 (DIAPH1) controls the actin polymerization. Taperin (TPRN) modulates actin dynamics through direct or indirect contact with the ends of actin filaments. Chloride intracellular channel 5 (CLIC5) stabilizes membrane-actin filament linkages at the base of hair cell stereocilia as part of a molecular complex with radixin (RDX), TPRN, and myosin VI (MYO6). The protein tyrosine phosphatase receptor type Q (PTPRQ) hydrolyzes 4,5-phosphatidylinositol bisphosphate (PIP2), a key regulator of actin remodeling. TRIO and the F-actin binding protein (TRIOBP) stabilizes F-actin structures. Finally, when the core of the actin filament known as actin gamma 1 (ACTG1) is altered, the autosomal dominant form of hearing loss develops.

Dysfunction in cell-cell adhesion protein complexes also may cause instances of autosomal recessive deafness. Otogelin (OTOG) and otoancorin (OTOA) are important proteins for the attachment of acellular gels to the underlying nonsensory cells in the inner ear. The MARVEL domain containing 2 (MARVELD2), tight junction protein 2 (TJP2), and claudin 14 (CLDN14) together provide regular tight junction assemblies. A carcinoembryonic antigen-related cell adhesion molecule 16 (CEACAM16) on the tips of the higher stereocilia and the tectorial membrane (TM) protein alpha-tectorin (TECTA) are essential for maintaining the integrity of the tectorial membrane and for the association of stereocilia with the TM. Dysfunctional CEACAM16 or TECTA cause autosomal dominant nonsyndromic deafness and a recessive form of sensorineural prelingual nonsyndromic deafness (TECTA).

Solute carrier family 26 (anion exchanger) member 5 (SLC26A5, also known as prestin) shuttles chloride ions across the cell membrane and undergoes a conformational change in response to changes in intracellular Cl levels leading to electromotility of outer hair cells.

The cadherin-related 23 (CDH23) protein and protocadherin-related 15 (PCDH15) play a major role in forming a tip link between the top of a shorter stereocilium and the side of the nearby taller stereocilium. The tension exerted on the tip of the lower stereocilium after the sound stimulation allows K^+ to enter the hair cells via the mechanoelectrical transducer (MET) channel on membranes of the lower stereocilium. Transmembrane channel like 1 and 2 (TMC1 and TMC2), tetraspan transmembrane protein hair cell stereocilia (LHFPL5), protocadherin-related 15 (PCDH15), and transmembrane inner ear (TMIE) proteins are likely to be involved in the organization of MET channels, although the channel's exact molecular

composition is not known. A *TMIE* mutation is associated with autosomal recessive nonsyndromic hearing loss, the most common form of congenitally acquired hearing impairment. *TMC1* variations are related to progressive postlingual hearing loss and profound prelingual deafness.

Loss-of-function mutations in several genes coding myosins and cell adhesion proteins are responsible for the development of the rare congenital disorder known as Usher syndrome. These include the molecular motor myosin VIIa (*MYO7A* also known as *USH1B*), cell-cell adhesion cadherin proteins *CDH23* (also known as *USH1D*) and *PCDH15* (also known as *USH1F*), the scaffold proteins USH1 protein network component sans (*USH1G*) and USH1 protein network component harmonin (*USH1C*) genes, and genes coding the proteins Usher syndrome 2A (*USH2A*) and deafness autosomal recessive 31 (*DFNB31* also known as *USH2D*). Functional alterations in the calcium and integrin binding family member 2 (CIB2) protein lead to the development of Usher syndrome type 1J and nonsyndromic deafness. And, finally, polymorphisms in *CDH23*, *PCDH15*, *MYO15A*, or *MYO6* predispose to age-related hearing loss (Ahmed et al., 2013; Azaiez et al., 2015; Brownstein et al., 2014; Cosgrove and Zallocchi, 2014; El-Amraoui and Petit, 2005; Hwang et al., 2012; Jiang et al., 2014; Kammerer et al., 2012; Kremer et al., 2006; Op de Beeck et al., 2011; Pan and Zhang, 2012; Reiners et al., 2006; Schwander et al., 2010; Verpy et al., 2011; Yan and Liu, 2010).

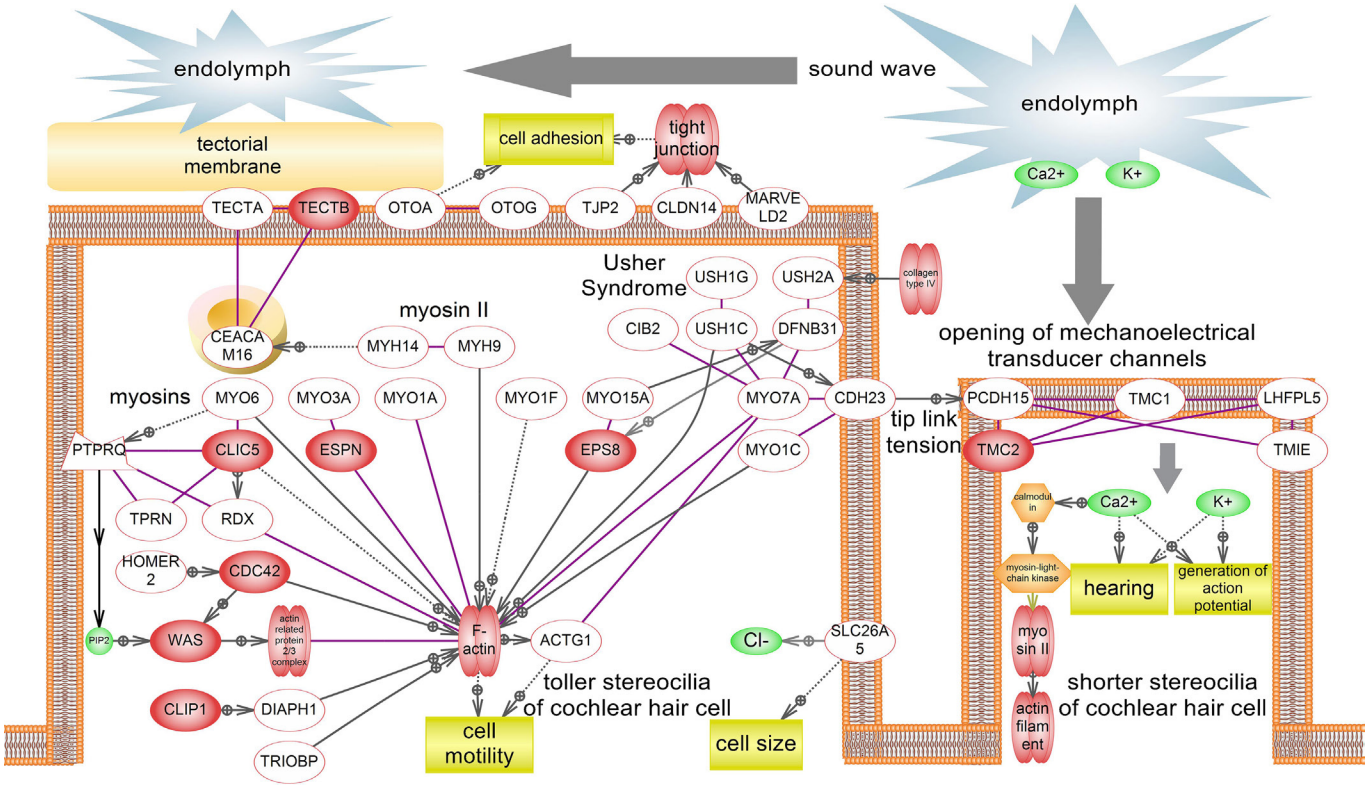

FIG. 1 Pathway 1: Dysfunction of cochlear hair cell stereocilia proteins in hearing loss.

Pathway 2

Dysfunctions of cochlear hair cell synapse proteins in hearing loss (Fig. 2)

Incoming signals

Hearing depends on neurotransmission from the cochlear hair cells to the peripheral axon of the spiral ganglion neuron through the glutamatergic synapse. Some genes, encoding proteins implicated in synaptogenesis, may be mutated and exhibit diminished functions in the congenital hearing loss. Those genes include otoferlin (*OTOF*), GIPC PDZ domain containing family member 3 (*GIPC3*), solute carrier family 17 (vesicular glutamate transporter), member 8 (*SLC17A8*), calcium voltage-gated channel subunit alpha1 D (*CACNA1D*), and myosin VI (*MYO6*).

Outcome effects

Due to dysfunctions of these proteins, the glutamatergic synapse between cochlear hair cells and peripheral axon of spiral ganglion neuron neurotransmission is impaired resulting in hearing loss (Charizopoulou et al., 2011; Cosgrove and Zallocchi, 2014; Friedman et al., 2009; Gregory et al., 2013; Heidrych et al., 2009; Luo et al., 2013; Moser et al., 2013; Newman et al., 2012; Pan and Zhang, 2012; Reiners et al., 2006; Roux et al., 2006; Yan and Liu, 2010; Zallocchi et al., 2012).

Signaling

The neurotransmitter glutamate needs to be loaded into synaptic vesicles before it is released into the synaptic cleft. The glutamatergic ribbon synapses of hair cells use the vesicular glutamate transporter SLC17A8 (also known as VGLUT3) to load their synaptic vesicles with glutamate. Mutations in the *SLC17A8* gene cause autosomal dominant nonsyndromic deafness.

Unlike in other synapses, hair cell ribbon synapses use CACNA1D (CaV1.3 L-type Ca^{2+} channels) to stimulate glutamate secretion. The calcium-binding protein 2 (CABP2) might play a role in regulating CACNA1D and therefore inner hair cell synaptic transmission. A loss-of-function mutation in the *CACNA1D* gene has been linked to familial congenital deafness and bradycardia. Variations in the *CABP2* gene were associated with moderate sensorineural hearing impairment.

Mutations in OTOF cause both prelingual deafness and temperature-sensitive synaptic hearing impairment. OTOF binds Ca^{2+} during the hair cell glutamate exocytosis and may substitute for the classic synaptic fusion proteins synaptotagmins (SYT1 or SYT2). OTOF

supports Ca^{2+}-dependent interactions with syntaxin 1A (STX1A) and the synaptosome-associated protein 25 kDa (SNAP25). MYO6 was shown to be a novel OTOF-binding partner.

Mutations in other genes that play a role in vesicle exocytosis in cochlear hair cells have been associated with hearing loss. GIPC3 may take part in Ca^{2+}-dependent exocytosis in cochlear hair cells. PCDH15 and the adhesion G protein–coupled receptor V1 (ADGRV1) complex may connect with SNAP25 to control vesicle docking and fusion in synaptosomes from the organ of Corti. The absence or loss of function of one of the components of the complex results in a delay in synaptic maturation.

Finally, polymorphisms of the glutamate metabotropic receptor 7 (*GRM7*) gene are a significant risk factor for age-related hearing loss development. GRM7 activation inhibits the cyclic adenosine monophosphate (cAMP) cascade and synaptic glutamate exocytosis by providing negative feedback upon glutamate release. The lack of GRM7 function leads to neuronal damage due to glutamate excitotoxicity resulting in hearing loss.

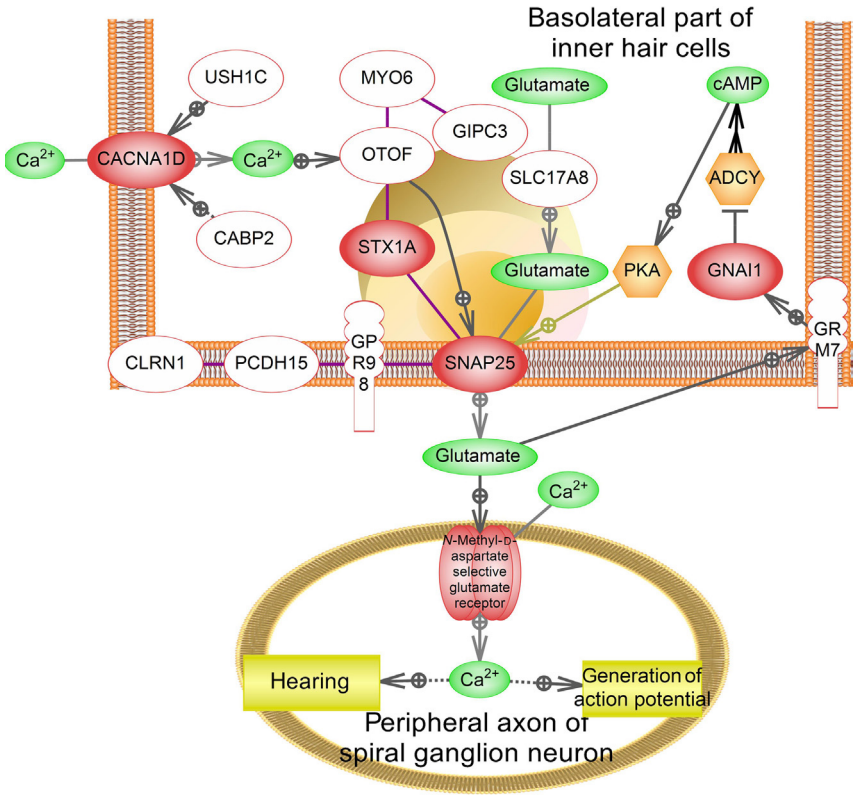

FIG. 2 Pathway 2: Dysfunction of cochlear hair cell synapse proteins in hearing loss.

Pathway 3

Impairment of mechanoelectrical transduction and potassium cycling in the inner ear in hearing loss (Fig. 3)

Incoming signals

The cochlear canals contain two types of fluid: perilymph and endolymph. Perilymph has an ionic composition similar to extracellular fluid found elsewhere in the body (i.e., it is K^+-poor and Na^+-rich), and it fills the scalae tympani and vestibule. Hearing depends on the high K^+ concentration in endolymph that bathes the apical membranes of sensory hair cells. K^+ enters the hair cell through mechanoelectrical transducer channels. K^+ ions exit from hair cells, transfer between endolymph and perilymph, and are recycled by Deiter cells, fibrocytes, and marginal cells of the stria vascularis. Dysfunctions in the proteins involved in mechanoelectrical transduction and K^+ recycling cause hearing loss.

Outcome effects

Dysfunctional proteins of mechanoelectrical transducer channel and K^+ channels impair the K^+ circulation in endolymph of the inner ear and the transduction of sound waves into neuronal signals normally produced by action potential generation in hair cell membrane.

Signaling

When stereocilia on cochlear hair cells move, mechanoelectrical transducer channels open, and K^+ enters the hair cell via apical MET channels. Mutations in the genes coding the MET channels (*TMC1*, *TMC2*, *LHFPL5*, *TMIE*, and *PCDH15*) are associated with different forms of congenital deafness (see Pathway 1).

When K^+ enters through the hair cell membrane, depolarization occurs. Depolarization in turn opens voltage-gated calcium channels (i.e., the purinergic receptor P2X 2 (P2RX2), transient receptor potential cation channel subfamily C member 1 (TRPC1), and the ATPase plasma membrane Ca^{2+} transporting 1 and 2 (ATP2B2 and ATP2B2)) in the hair cell membrane to stimulate Ca^{2+} influx and cause glutamate release from the basal end of the cell onto the auditory nerve endings (see Pathway 2). *P2RX2* mutations are associated with autosomal dominant nonsyndromic hearing loss.

K^+ exits from the hair cells through the potassium voltage-gated channel subfamily Q member 4 (KCNQ4) and the potassium calcium-activated

channel subfamily M alpha 1 (KCNMA1) channels. *KCNQ4* mutations are found in patients with nonsyndromic sensorineural deafness type 2, an autosomal dominant form of progressive hearing loss.

Supporting Deiters cells take K^+ back via potassium voltage-gated channel subfamily J member 10 (KCNJ10), and it is exported out by solute carrier family 12 (potassium/chloride transporters) and member VI and VII (SLC12A6 and SLC12A7). Mutations in *KCNJ10* cause the autosomal recessive EAST syndrome characterized by epilepsy, ataxia, sensorineural deafness, and a salt-wasting tubulopathy. Polymorphisms in the *KCNQ4* gene are strongly associated with several types of hearing loss including autosomal recessive EAST syndrome. The knockout of either the *SLC12A6* or *SLC12A7* genes causes deafness in mice.

K^+ passes between fibrocytes of the lateral wall through gap junctions. At least three connexin genes (gap junction protein beta *GJB2*, *GJB3*, and *GJB6*) belong to the gap junction system and are involved in congenital deafness. Mutations in *GJB2* (the variation 35delG is the most common one) are responsible for as much as 50% of prelingual, recessive deafness.

In strial vascularis marginal cells, the solute carrier family 12 (sodium/potassium/chloride transporter) member 2 (SLC12A2 also known as NKCC1), ATPase Na^+/K^+ transporting subunit alpha 1 and 2 (ATP1A1 and ATP1A2) raise the intracellular K^+ concentration. In parallel the chloride voltage-gated channel Ka/barttin CLCNK type accessory beta subunit (CLCNKA/BSND) and chloride voltage-gated channel Kb/barttin CLCNK type accessory beta subunit (CLCNKB/BSND) channels recycle Cl^-. BSND is thought to be an accessory subunit of a chloride channel, and if mutated, it disrupts the activity of CLCNKA and CLCNKB. Mutations in the *BSND* gene are associated with Bartter syndrome leading to sensorineural deafness (Bartter syndrome type IV). Further, K^+ exits through apical channels, specifically the potassium voltage-gated channel subfamily Q member 1 (KCNQ1) and potassium voltage-gated channel subfamily E regulatory subunit 1 (KCNE1) back into the endolymph. Mutations in the *KCNE1* and *KCNQ1* genes cause Jervell and Lange-Nielsen syndrome (long QT syndrome, associated with a bilateral sensorineural hearing loss). Interestingly, *SLC12A2*, *ATP1A1*, and *ATP1A2* heterozygous deletions were shown to cause an age-dependent hearing loss in mice (Chen and Zhao, 2014; Hibino and Kurachi, 2006; Janssen et al., 2009; Lang et al., 2007; Mahdieh and Rabbani, 2009; Naito et al., 2013; Nie, 2008; Sliwinska-Kowalska and Pawelczyk, 2013; Tian et al., 2007; Van Eyken et al., 2006, 2007; Wang et al., 2014; Zhang et al., 2014).

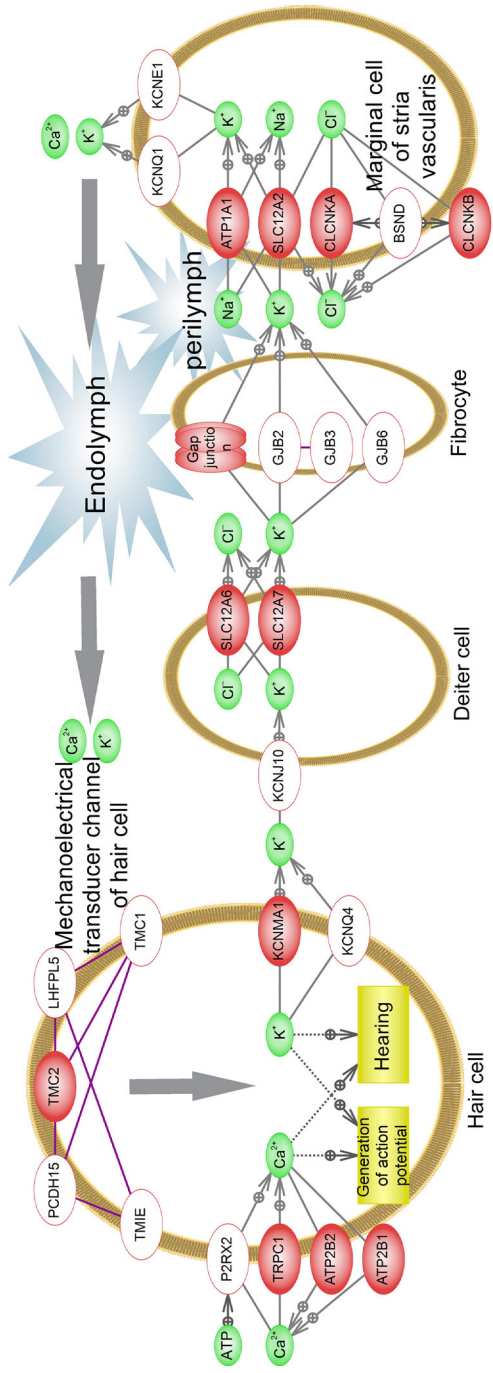

FIG. 3 Pathway 3: Deficiency of potassium cycling in hearing loss.

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Disease numbers # 609533, # 601067, # 601386, # 612976, # 606346, # 601071 (and many others) in Online Mendelian Inheritance in Man (OMIM database, www.omim.org).

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CHAPTER

7.2

Otitis media

Otitis media is defined as an infection of the middle ear fluid and is the second most common pediatric diagnosis in the emergency department following upper respiratory infections. Although otitis media can occur at any age, it is most commonly seen between the ages of 6–24 months.

Otitis media is the rapid onset of signs and symptoms of inflammation in the middle ear. ***(Ferri and Ferri, 2018)***.

Infection of the middle ear can be viral, bacterial or a coinfection with both. The most common etiologic factor is a viral upper respiratory tract infection, which causes inflammation and dysfunction of the eustachian tube leading to the transient aspiration of nasopharyngeal secretions into the middle ear. The most common viral pathogens of otitis media include the respiratory syncytial virus (RSV), coronaviruses, influenza viruses, adenoviruses, human metapneumovirus, and picornaviruses (Danishyar and Ashurst, 2018). Bacterial colonization from the nasopharynx in conjunction with eustachian tube dysfunction also leads to the infection. The most common bacteria that cause otitis media are *Streptococcus pneumoniae* (*S. pneumoniae*), followed by Nontypeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis*. *S. pneumoniae* causes from 30% to 40% of all cases of otitis media. The second most common bacterial pathogen is *H. influenzae*, which causes up to 50% of cases. *M. catarrhalis* causes the last proportion of 10%–20% of cases. Infection caused by penicillin-nonsusceptible *S. pneumoniae* (PNSSP) (MIC > 0.1 mg/mL) becomes the infection of increasing importance ranging from 8% to 34% of all otitis media cases. About 50% of PNSSP isolates are penicillin intermediate (with MIC of 0.1–2.0 mg/mL) (Ferri and Ferri, 2018).

The epithelial cells of the middle ear contain several defense mechanisms including (1) the presence of mucous glycoproteins and surfactants, which trap infectious agents; (2) the ability to secrete defense molecules such as the defensins or interferons; and (3) increased antibody production through the adaptive immune response.
The low level of activity of Toll-like receptor (TLR) signaling in epithelial cells in the human middle ear decreases the secretion of defense

molecules and cytokines by epithelial cells, which in turn is needed for activation of cells of immune system:

Pathway 1. *Insufficient activation of immune response in the middle ear epithelium cells in otitis media* (Fig. 4).

Pathogens also stimulate extra mucus production in the middle ear, however, which further complicate the reduction of inflammation characteristic of otitis media.

Pathway 2. *Pathogens stimulate mucins expression in the middle ear* (Fig. 5).

Key cellular contributors and processes

Extracellular matrix proteins
Protein or gene

The extracellular matrix (ECM), an essential component of most tissues in multicellular organisms, is a noncellular network of macromolecules secreted by the surrounding cells. The ECM provides structural support to the tissue and is strongly involved in intercellular signaling.

Middle ear
Anatomic structure

Middle ear is the internal part of the ear that conducts sound from the outer to the inner ear.

Mucus
Process

Mucus is a heterogeneous mixture of secreted polypeptides (termed mucins), cells, and cellular debris that may tether together at the fluid surface by oligomeric mucin protein complexes.

NOD-like receptors
Protein or gene

The NOD-like receptors (nucleotide-binding oligomerization domain-like receptors, NLRs) are cytoplasmic pattern recognition receptors. NLRs can bind to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) inside the cell and have a variety of functions in the regulation of inflammatory and apoptotic responses. The NLR family consists of several proteins divided into subfamilies based on their N-terminal protein-interacting domains.

Toll-like receptors
Protein or gene

Toll-like receptors belong to a family of membrane proteins that can directly bind microbial molecules or proteins and initiate the innate immune response.

Pathway 1

Insufficient activation of immune response in the middle ear epithelium cells in otitis media (Fig. 4)

Incoming signals

Low expression of pattern recognition receptors leads to insufficient immune response in the middle ear epithelium cells in otitis media. Middle ear epithelial cells express all types of pattern recognition receptors such as the Toll-like receptors (TLRs), cytoplasmic nucleotide-binding oligomerization domain (NOD)-like receptors, C-type lectin receptors, and retinoic acid–inducible genes (*DDX58* (DExD/H-box helicase 58)). TLR signaling provides protection against infection by recognizing intruding pathogens through their invariant pathogen-associated molecular patterns and mobilizing appropriate immune system response. Patients with chronic middle ear disease have been shown to exhibit lower mRNA and protein levels for TLR2, TLR4, TLR5, TLR7, and TLR9 compared with a control group.

Outcome effects

The downregulation of TLRs, NODs, and other pattern recognition receptor expression in otitis media leads to an inefficient defense in the middle ear, which in turn causes repeated infections and persistent inflammations.

Signaling

TLRs can sense pathogens through their pathogen-associated molecular patterns. Among others, TLR3 recognizes dsRNA, TLR2 and TLR4 recognize bacterial lipopolysaccharides (LPS), TLR5 responds to bacterial flagellin, TLR7/8 mediates recognition of ssRNA, and TLR9 recognizes the CpG sites of bacterial and viral DNA. Also, proteins derived from *H. influenzae* serve as ligands for TLR2 in otitis media. A lipooligosaccharide (LOS), which is expressed on mucosal Gram-negative bacteria, serves as a ligand for both TLR2 and TLR4. Proteins specific for *S. pneumoniae* are considered ligands for TLR4.

The activation of most TLRs results in downstream activation of the myeloid differentiation primary response 88 (MyD88) gene, which in turn activates the interleukin 1 receptor–associated kinase (IRAK1–IRAK4) and TNF receptor–associated factor 6 (TRAF6) cascades. Then, activation of MAPKs and transcription factors (primary NF-κB and JUN/FOS) occurs

leading to the expression of proinflammatory proteins and the stimulation of immune responses.

When pathogens bypass the membrane-associated pattern recognition receptors, they encounter cytoplasmic pattern recognition receptors such as the nucleotide-binding oligomerization domain containing 1,2 (NOD1,2), interferon induced with helicase C domain 1 (IFIH1), and DExD/H-box helicase (58DDX58) proteins. NOD1 and NOD2 initiate immune responses through the formation of inflammasomes, and they activate NF-κB, leading to the production of inflammatory cytokines. Patients with otitis media have significantly lower levels of expression of NOD1 and NOD2, as well as DDX58. The development of recurrent otitis media may be associated with these decreased expression levels, demonstrating the protective roles of NOD1, NOD2, and DDX58 against ear infections.

As a result of insufficient activation, TLR and NOD signaling in middle ear epithelium does not promote the release of enough cytokines, interferons, and other defensive proteins.

For example, BPI fold containing family A member 1 (BPIFA1) and DEFB4A (human beta-defensin 2) have broad-spectrum antimicrobial activity. They reduce bacterial biofilm formation by *Pseudomonas aeruginosa*. BPIFA1 also acts as a chemoattractant that recruits macrophages and neutrophils to the site of infection. It has been found that BPIFA1 is essential in the maintenance of middle ear fluid pressure and efficient mucociliary clearance.

Middle ear epithelial cells also produce lysozyme, an antimicrobial molecule of innate immunity that degrades the peptidoglycans found in bacterial cell walls. Lysozyme and DEFB4A have synergistic effects against *S. pneumoniae* in otitis media (Chen et al., 2004; Granath et al., 2011; Hirano et al., 2007; Kim et al., 2010, 2014; Lee et al., 2008, 2013; Mittal et al., 2014; Moon et al., 2006; Philpott et al., 2014; Shimada et al., 2008; Si et al., 2014).

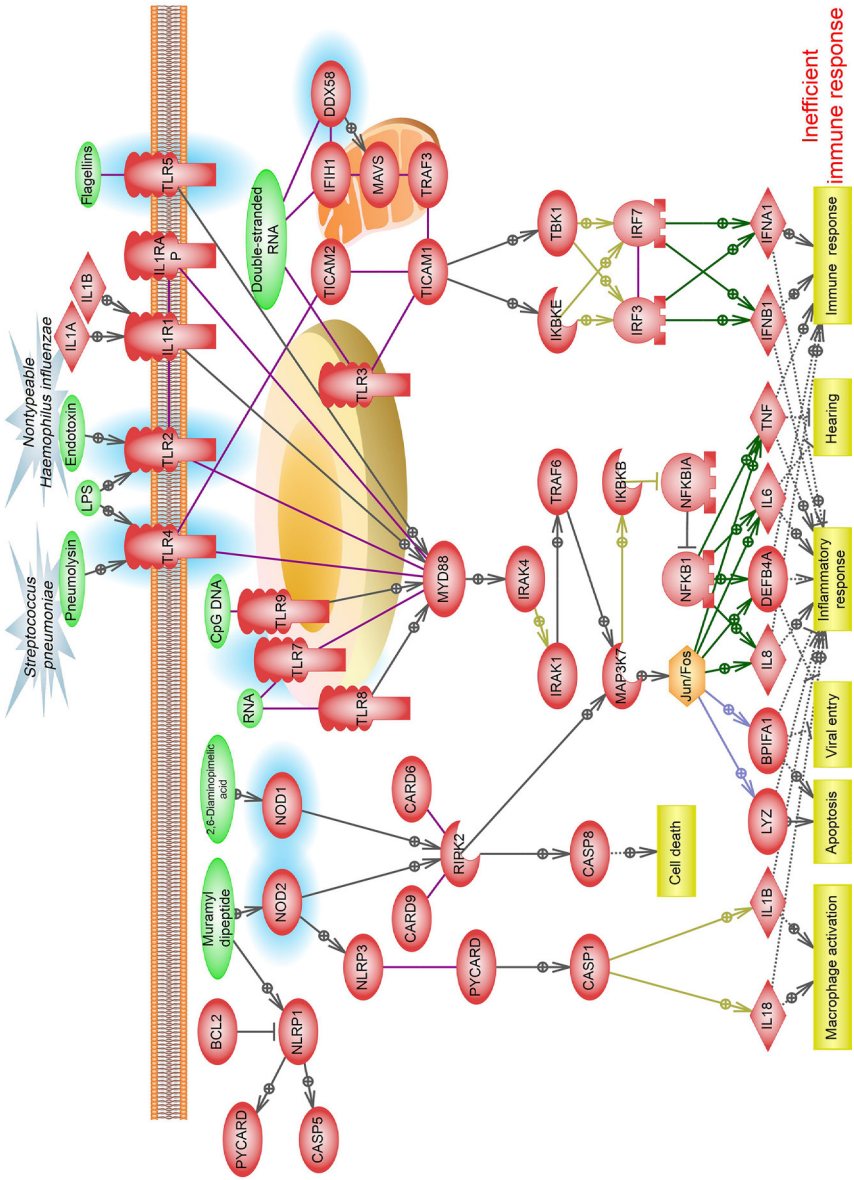

FIG. 4 Pathway 1: Insufficient activation of immune response in the middle ear epithelium cells in otitis media.

Pathway 2

Pathogens stimulate mucins expression in the middle ear (Fig. 5)

Incoming signals

The Gram-positive bacterium *S. pneumoniae*, Gram-negative bacteria nontypable *H. influenza* (NTHi) and *M. catarrhalis* synergistically induce the activation of mucus production in the middle ear effusion of patients with chronic otitis media. The viscous mucus of the middle ear is a heterogeneous mixture of secreted polypeptides, mainly mucins. The rise of mucin production is a vital defense response against invading microbes (also see Asthma). Excess mucin production, however, results in a conductive hearing loss observed in otitis media.

Outcome effects

Abnormally generous amount of viscous mucus in the middle ear prevents active mucociliary clearance in otitis media. The overproduction of MUC2 (mucin 2, oligomeric mucus/gel-forming), MUC5AC (mucin 5AC), and MUC5B (mucin 5B) by epithelial cells obstructs the transmission of sound waves from the middle ear to the inner ear.

Signaling

Pathogens adhered to host epithelial cells stimulate the activation of TRL pathways. Polymorphisms in the gene encoding *TLR4* have been associated with recurrent acute otitis media. Pneumolysin, endotoxin, and lipopolysaccharides are typical trigger signals produced by *S. pneumoniae*, NTHi, and *M. catarrhalis*, respectively. These ligands induce mucin (MUC5AC, MUC5B, and MUC2) expression through the activation of the MyD88-MAP3K7 and MAP3K1 (mitogen-activated protein kinase kinase kinase 7 and 1) cascade. The activation of MAPKs is also required for the synergistic induction of mucin expression by pathogens.

Also, *S. pneumoniae* works synergistically with NTHi to induce mucin expression via an AP1-dependent mechanism. Gram-negative NTHi and *S. pneumoniae* synergistically induce activation of major AP-1 subunits including activating transcription factor 2 (ATF-2) and JUN (Jun proto-oncogene, AP-1 transcription factor subunit).

Epidermal growth factor receptor (EGFR) signaling is also involved in the activation of the JUN and FOS (Fos proto-oncogene, an AP-1 transcription factor subunit) transcription factors and leads to mucin synthesis.

Interleukin-1B (IL-1B) and tumor necrosis factor (TNF) signals stimulate mucin expression via canonical NF-kb activation. The canonical NF-kb

pathway is initiated by TNF via its cognate receptor (TNFR1) and by IL-1 via the IL-1 receptor (IL-1R) (Bhutta et al., 2017; Cho et al., 2016; Elsheikh and Mahfouz, 2006; Emonts et al., 2007; Ha et al., 2008; Hernandez et al., 2015; Kawano et al., 2000; Kerschner, 2007; Kerschner et al., 2010; Leichtle et al., 2009; MacArthur et al., 2011; Preciado et al., 2010; Shen et al., 2008; Ubell et al., 2008).

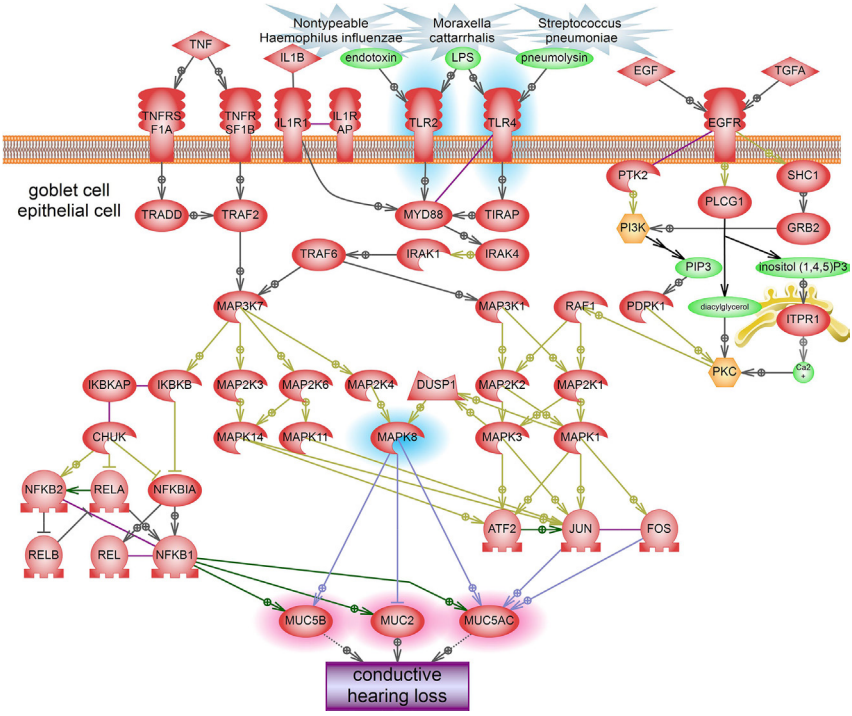

FIG. 5 Pathway 2: Pathogens stimulate mucins expression in the middle ear.

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