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Hearing Loss Associated with Enlargement of the Vestibular Aqueduct: Mechanistic Insights from Clinical Phenotypes, Genotypes, and Mouse Models

Andrew J. Griffith[1,*] and **Philine Wangemann**[2]

[1] Otolaryngology Branch, National Institute on Deafness and Other Communication Disorders, 5 Research Court, Rockville, Maryland 20850-3320 USA

[2] Anatomy and Physiology Department, 205 Coles Hall, Kansas State University, Manhattan, Kansas 66506 USA

Abstract

Enlargement of the vestibular aqueduct (EVA) is one of the most common inner ear malformations associated with sensorineural hearing loss in children. The delayed onset and progressive nature of this phenotype offer a window of opportunity to prevent or retard progression of hearing loss. EVA is not the direct cause of hearing loss in these patients, but rather is a radiologic marker for some underlying pathogenetic defect. Mutations of the *SLC26A4* gene are a common cause of EVA. Studies of an *Slc26a4* knockout mouse demonstrate that enlargement of the scala media is a key event in the pathogenesis of deafness. The enlargement is driven by fluid secretion in the vestibular labyrinth and a failure of fluid absorption in the embryonic endolymphatic sac. Elucidating the mechanism of hearing loss may offer clues to potential therapeutic strategies.

1. Clinical Phenotypes Associated with EVA

Enlargement of the vestibular aqueduct (EVA) is a common malformation identified in ears of children undergoing high-resolution imaging for sensorineural hearing loss (Fig. 1A). An enlarged vestibular aqueduct is also sometimes referred to as a dilated or large vestibular aqueduct (DVA or LVA). Valvassori and Clemis established the modern radiologic definition of EVA as a midpoint diameter of >1.5 mm or a grossly malformed overall morphology (Valvassori et al., 1978). These criteria have been adopted by a majority of studies. Computed tomography (CT) is the best radiologic modality to image bony structures such as the vestibular aqueduct. A single axial CT section can show the full length of the J-shaped vestibular aqueduct coursing from its aperture on the posterior aspect of the temporal bone to the medial aspect of the vestibule. The normal vestibular aqueduct is often so narrow that it is not visible in CT images. Magnetic resonance (MR) imaging provides complementary visualization of the soft tissue and fluid contents of an enlarged vestibular aqueduct: an enlarged endolymphatic sac and duct (Fig. 1B) (Phelps et al., 1998). The relationship of the vestibular aqueduct with the endolymphatic duct and sac is shown in Fig. 2.

[*]Corresponding author: griffita@nidcd.nih.gov; Tel. 301.402.2686; FAX, 301.402.7580.

Two studies published in 1989 described a distinctive auditory phenotype associated with isolated EVA (Jackler et al., 1989; Levenson et al., 1989). The hearing loss is predominantly sensorineural, variable in severity, asymmetric or unilateral, with a pre- or peri-lingual onset (before or near the time of speech and language acquisition). Many EVA patients have evidence of a conductive hearing loss component associated with normal middle ear findings (Arjmand et al., 2004; Govaerts et al., 1999; Nakashima et al., 2000). This is thought to be a cochlear conductive hearing loss due to a “third window” effect of the EVA upon sound transmission within the labyrinth (Merchant et al., 2007).

The sensorineural hearing loss associated with EVA can fluctuate or progress in a stepwise incremental fashion (Jackler et al., 1989; Levenson et al., 1989). In some patients, sudden hearing loss can be precipitated by minor head trauma or barotrauma. Although original reports emphasized EVA as the sole radiologic abnormality in these ears, this phenotype may also be observed in ears with EVA and cochlear anomalies. Associated cochlear anomalies can include a “Mondini” cochlea with reduced number of cochlear turns and an incomplete osseous partition of the turns. A more commonly observed anomaly in EVA ears is a hypoplastic cochlear modiolus (Lemmerling et al., 1997). There are differing conclusions on whether the presence or absence of cochlear malformations is related to the severity of hearing loss (Azaiez et al., 2007). However, in a study in which other underlying genotypic and phenotypic correlations were statistically accounted for, the presence of an associated cochlear anomaly was not independently associated with severity of hearing loss in ears with EVA (King et al., 2010).

The delayed onset and progressive nature of hearing loss associated with EVA provides a therapeutic window for interventions to prevent or slow the progression of hearing loss. Such strategies could be of particular benefit during the critical period of speech and language acquisition in young children. Current strategies for patients with EVA include counseling to avoid head trauma and barotrauma, and rehabilitation of communication. The latter can be achieved with conventional hearing amplification or cochlear implantation according to the degree of hearing loss. Corticosteroids have been used to treat hearing loss associated with EVA (Lin et al., 2005). The results of these studies are difficult to interpret because the natural history of hearing loss associated with EVA is unpredictable and idiosyncratic. Rigorous clinical trials will be required to evaluate these or other interventions for EVA.

The distinctive hearing loss phenotype associated with EVA has spawned a variety of hypotheses for the mechanism of hearing loss. One early theory proposed that trauma or barotrauma increases intracranial pressure with reflux of the contents of the endolymphatic sac and duct into the scala media where it damages hair cells and hearing (Jackler et al., 1989). This theory lost favor because operations to obliterate or decompress the endolymphatic sac were either ineffective or detrimental to hearing (Wilson et al., 1997). Furthermore, there is no correlation of the size of the EVA with hearing loss (Griffith et al., 1996; King et al., 2010). A second theory proposed that hearing loss results from leakage of perilymph from an abnormal fistulous round window (Belenky et al., 1993), but this observation has not been reported by other authors. There are phenotypic similarities between EVA and the fluctuating hearing loss thought to be associated with endolymphatic hydrops, in which endolymph-containing spaces are dilated. Endolymphatic hydrops has been proposed to cause hearing loss via increased endolymph osmotic pressure, rupture of intracochlear membranes, alterations of endolymph composition due to mixing with perilymph, and damage to hair cells and hearing. However, recent analyses cast doubt on this mechanism of hearing loss and suggest that endolymphatic hydrops is a nonspecific marker for an underlying cellular or molecular lesion that is the direct cause of hearing loss

(Merchant et al., 2005). Similarly, EVA is not thought to be a direct cause of hearing loss, but a radiologic marker for an underlying molecular or cellular defect (Griffith et al., 1996).

2. Genetics of EVA

EVA with hearing loss typically presents as a sole clinical abnormality, in which case it is termed nonsyndromic. EVA has been reported in association with congenital cytomegalovirus (CMV) infection (Bauman et al., 1994), which can cause a similar hearing loss phenotype (Dahle et al., 2000). However, congenital CMV infection is not a significant or common cause of EVA (Pryor et al., 2005a). EVA may also be associated with abnormalities of other organ systems as part of a genetic syndrome. Examples of syndromes that can include EVA are distal renal tubular acidosis with deafness, CHARGE syndrome, Waardenburg syndrome, and branchio-oto-renal syndrome. However, the most common syndrome associated with EVA is Pendred syndrome. Pendred syndrome has been phenotypically estimated to account for up to 10% of cases of hereditary hearing loss (Fraser, 1965).

Pendred syndrome is an autosomal recessive disorder that was originally described in 1896 as a combination of goiter (thyroid gland enlargement) and severe congenital deafness (Pendred, 1896). We now realize the phenotypic spectrum of Pendred syndrome is much broader. Although goiter is incompletely penetrant, there is an underlying, more penetrant, defect in the ability of the thyroid gland to organify iodine (i.e. incorporate inorganic iodine in thyroid hormone biosynthesis) (Morgans et al., 1958; Pryor et al., 2005b). The hearing loss is often milder and more delayed in onset than originally described and, in some cases, may even be unilateral. An important advance was recognition that EVA is a highly penetrant feature of Pendred syndrome (Phelps et al., 1998). Due to routine hearing screening and radiologic imaging of the temporal bones, Pendred syndrome now commonly presents as nonsyndromic EVA in children (Reardon et al., 2000). Vestibular dysfunction is incompletely penetrant and varies in severity from subclinical caloric hyporeflexia to severe vertiginous episodes (Bergstrom, 1980; Das, 1987).

Mutations in the *SLC26A4* gene (formerly known as *PDS*) cause Pendred syndrome (Everett et al., 1997). Mutations in *SLC26A4* can also be detected in some patients with nonsyndromic EVA (Usami et al., 1999). This genotypic and phenotypic overlap has caused confusion about the nosologic relationship of these disorders. Some authors consider Pendred syndrome and nonsyndromic EVA to be variants of the same disorder (Campbell et al., 2001) while others regard them as distinct entities based upon *SLC26A4* genotypic and phenotypic correlations (Pryor et al., 2005b).

Only one fourth of North American Caucasian EVA patients have two detectable mutant alleles of *SLC26A4*, one fourth have one detectable mutant allele, and one half of patients have no mutations (Campbell et al., 2001; Choi et al., 2009c). The causes of EVA in patients with only one or zero mutations of *SLC26A4* are unknown. Undetected large genomic deletions or cryptic mutations in noncoding regions do not appear to account for this observation (Choi et al., 2009b). Digenic inheritance with mutations in the *FOXI1* or *KCNJ10* genes has been proposed for patients with one *SLC26A4* mutation (Pryor et al., 2005b; Yang et al., 2007; Yang et al., 2009), but these findings have not been replicated in other studies (Jonard et al., 2010; Pera et al., 2008; Wu et al., 2010) and alternative hypotheses have not been excluded (Choi et al., 2009a). Mendelian genetic factors are unlikely in most EVA patients with no mutations since the proportion of siblings with EVA is much less than predicted for an autosomal recessive trait (Campbell et al., 2001; Choi et al., 2009c). In other populations such as Koreans, two mutant alleles can be found in 81% of EVA patients (Park et al., 2005).

In some of the reported genotypic surveys of childhood deafness among different populations, *SLC26A4* mutations are the most common known genetic cause of childhood deafness (Anwar et al., 2009; Park et al., 2003). Genotypic surveys of large study populations have indicated that *SLC26A4* mutations account for up to or more than 10% childhood deafness (Anwar et al., 2009; Park et al., 2003; Yuan et al., 2009). This comparatively high prevalence provides another impetus to develop new therapeutic or preventive strategies for EVA.

SLC26A4 encodes a multi-pass transmembrane protein called pendrin (Everett et al., 1997). Pendrin is expressed in a limited tissue distribution that includes the inner ear, thyroid, and kidney (Everett et al., 1997). Pendrin has been shown to exchange a variety of anions (Cl^- and I^-) and bases (e.g., OH^- and HCO_3^-) across apical plasma membranes of epithelial cells (Royaux et al., 2001; Scott et al., 1999; Soleimani et al., 2001). In the thyroid follicle, pendrin is thought to mediate the transport of inorganic iodine across the apical membranes of follicular cells into the follicular lumen for biosynthesis of thyroid hormone (Royaux et al., 2000). *In situ* functional studies of pendrin have largely utilized a targeted deletion allele ("knockout") of the mouse *Slc26a4* gene (Everett et al., 2001).

3. Pathophysiological mechanisms of hearing loss in EVA

Our most significant mechanistic insights into the pathogenesis of hearing loss associated with EVA are based upon the *Slc26a4* knockout (*Slc26a4*$^{-/-}$) mouse that segregates a targeted deletion of exon 8 of *Slc26a4* (Everett et al., 2001). Other mouse models include the *Foxi1* knockout mouse (Hulander et al., 2003) and the *loop* mouse line segregating a chemically induced mutation of *Slc26a4* (Dror et al., 2010).

The pathogenesis of EVA begins during the embryonic development of the inner ear. The inner ear develops from an invagination of the ectoderm that separates to form the initial otocyst. In mice the otocyst forms at embryonic day (E) 9.5 (Mansour et al., 2005). The otocyst is initially filled with amniotic fluid that has a plasma-like composition (Cheung et al., 2005). When and how the developing epithelia change the composition of the luminal fluid is currently unknown. At approximately E10.5, two protrusions begin to extend from the otocyst; one forms the cochlea and the other forms the endolymphatic sac. While the protrusions elongate and, in the case of the cochlea, coil, the center of the otocyst reorganizes into the vestibular labyrinth. The lumen of the cochlear protrusion opens at E14.5. Lumen formation depends on fluid secretion in the vestibular labyrinth and fluid absorption in the endolymphatic sac (Kim et al., 2010).

In the mouse inner ear, pendrin functions as a Cl^-/HCO_3^- exchanger (Wangemann et al., 2007). Pendrin is expressed in the cochlea, the vestibular labyrinth and the endolymphatic sac. In the endolymphatic sac, pendrin is expressed in mitochondrial-rich cells that are interspersed among the principal ribosomal-rich cells (Dou et al., 2004; Royaux et al., 2003; Wangemann et al., 2004). In the cochlea, pendrin is expressed in a spiraling sheet of outer sulcus and spindle cells located in the lateral wall. In the vestibular labyrinth, pendrin is expressed in sheets of transitional cells that surround sensory cell patches (Wangemann et al., 2004). The earliest expression of pendrin occurs in the endolymphatic sac at E11.5 (Kim et al., 2011). Expression in the endolymphatic sac increases rapidly at E14.5. The onset of expression in the cochlea, utricle and saccule occurs at E13.5 to E16.5 (Kim et al., 2011).

The initial pathologic alteration in *Slc26a4*$^{-/-}$ mice includes an enlargement of the endolymphatic sac and cochlea that develops at E14.5, which is three days after the failed onset of expression in the endolymphatic sac (Kim et al., 2011). The enlargement leads to an approximately 10-fold increase in the cross-sectional area of the cochlear lumen that parallels normal cochlear growth (Fig. 2). The second pathologic alteration is an

acidification of cochlear endolymph that develops at E15.5, which is one to two days after the failed onset of pendrin expression in the cochlea (Kim et al., 2011). Lack of pendrin expression also causes an acidification of the endolymphatic sac. However, this acidification develops later, at E17.5, which may reflect the stronger buffering power of the luminal fluid in the endolymphatic sac.

The enlargement and luminal acidification of the scala media spread the effect of pendrin deficiency from pendrin-expressing cells to a multitude of other cells. The enlargement may impair intercellular communication, possibly due to epithelial cell stretching and lengthening of diffusional distances between epithelial cells and between epithelial cells and mesenchymal cells such as fibrocytes. Intercellular communication plays a major role in cochlear development since impaired intercellular communication underlies the retarded development of the organ of Corti and may also contribute to the retarded development of stria vascularis (Kim et al., 2011; Wangemann et al., 2009). Thyroid hormone is a major factor in the retardation of the development of the organ of Corti. Fibrocytes located in the modiolus and in the lateral wall of the cochlea express, between P6 and P8, high levels of type 2 deiodinase (Dio2) to generate the biologically active hormone tri-iodothyronine from the prohormone thyroxine (Campos-Barros et al., 2000). Receptors for tri-iodothyronine are located in the organ of Corti and in other epithelial cells lining the cochlear duct (Bradley et al., 1994; Ng et al., 2009). The route taken by thyroid hormone between the hormone-generating cells and the receptor-bearing cells has not yet been delineated, although intercellular diffusion via gap junctions may be involved. Gap junctions may not only be the conduit for thyroid hormone but also for other growth and development-controlling factors as well as for nutritional substrates (Chang et al., 2008; Wang et al., 2009; Zhang et al., 2005). Lengthening of diffusion distances between fibrocytes and receptor-bearing epithelial cells may be responsible for the observed local hypothyroidism in *Slc26a4*$^{-/-}$ mice that leads to the observed retarded development of the organ of Corti (Wangemann et al., 2009).

The development of the stria vascularis in *Slc26a4*$^{-/-}$ cochleae is also retarded: the normal multilayered and highly vascularized anatomy is acquired with a delay (Kim et al., 2011). It is still unclear whether the retarded development of the stria vascularis in *Slc26a4*$^{-/-}$ mice is mainly a function of the approximately four-fold elevated H^+ concentration in endolymph (Wangemann et al., 2007) or a function of the enlargement that is associated with an approximately 2.5-fold stretching of epithelial cells, including strial marginal cells, and with a displacement of neighboring fibrocytes. The premature onset of connexin 26 expression in basal cells of the stria vascularis is consistent with an impaired coordination of strial development (Kim et al., 2011). At P10, the stria vascularis is affected by oxidative stress (Singh et al., 2008) and fails to establish a normal endocochlear potential (Wangemann et al., 2007). Oxidative stress leads to the loss of expression of the K^+ channel KCNJ10 protein, which is essential for the generation of the endocochlear potential (Singh et al., 2008; Wangemann et al., 2004). The endocochlear potential is essentially a K^+ equilibrium potential that is generated by KCNJ10 in the intermediate cells of the stria vascularis, in conjunction with the very low K^+ concentration of intrastrial fluid and a normally high K^+ concentration in the cytosol of intermediate cells (Marcus et al., 2002; Wangemann, 2006). It is unclear whether this oxidative stress is a function of insufficient expression of defense mechanisms or whether oxidative stress is due to higher rates of metabolism necessary to support higher rates of K^+ secretion to maintain a normal endolymphatic K^+ concentration in an approximately 10-fold larger volume of scala media (Royaux et al., 2003). In addition, the acidification of cochlear endolymph may contribute to the loss of the endocochlear potential by enhancing oxidative stress through acid-activation of the K^+ channel KCNQ1 (Unsold et al., 2000) and an increase in the rate of transepithelial K^+ secretion across stria marginal cells, which would be associated with an increase in metabolism (Singh et al., 2008). Indeed, the endocochlear potential is reduced by experimental maneuvers that lead to

an acute acidification of cochlear fluids (Ikeda et al., 1987a; Sterkers et al., 1984) and acidification of cochlear fluids has been shown to increase free radical stress, whereas alkalinization has a protective effect on hearing (Tanaka et al., 2004).

The luminal acidification and the loss of the endocochlear potential may jointly contribute to the approximately 100-fold elevation in the endolymphatic Ca^{2+} concentration (Ikeda et al., 1987b; Wangemann et al., 2007). Loss of the endocochlear potential may reduce the driving force for Ca^{2+} transport via cellular or paracellular pathways. Further, acidification inhibits transcellular Ca^{2+} absorption pathways that may include uptake of Ca^{2+} from endolymph via Ca^{2+}-permeable TRPV4 and TRPV5 channels and export into perilymph via Ca^{2+}-ATPases and Na^{+}/Ca^{2+} exchangers. TRPV4 and TRPV5 channels are expressed in multiple epithelial cells of the cochlea and are inhibited by a luminal acidification (Vennekens et al., 2001; Wangemann et al., 2007). The resulting inhibition of Ca^{2+} absorption may lead to a failure to establish the normal endolymphatic Ca^{2+} concentration of 22 μM (Bosher et al., 1978; Wangemann et al., 2007). This low endolymphatic Ca^{2+} concentration is critical for normal auditory function. Elevated Ca^{2+} concentrations reduce microphonic potentials generated by the sensory cells (Tanaka et al., 1980) and excessive Ca^{2+} concentrations may damage hair cells through Ca^{2+} overload. Sensory hair cells in *Slc26a4*$^{-/-}$ mice degenerate between P15 and P30 after a history of thyroid hormone deprivation and under the burden of an elevated luminal Ca^{2+} concentration, luminal acidification and a deficient endocochlear potential (Everett et al., 2001).

How might these observations in *Slc26a4*$^{-/-}$ mice explain the etiology of fluctuating hearing loss in EVA patients? It is conceivable that fluctuation is due to the sensitivity of the endocochlear potential to oxidative stress. The endocochlear potential and oxidative stress may comprise a negative feedback system that oscillates and generates fluctuations in the endocochlear potential, which is required for hearing (Fig. 4). The hypothesized feedback loop is comprised of three elements. First, reactive oxygen species (ROS) are generated by marginal cells of stria vascularis as a byproduct of metabolism, which is necessary to support K^{+} secretion (Wangemann et al., 1995). Second, the ROS-sensitive K^{+} channel KCNJ10 that generates the endocochlear potential and supplies K^{+} to the marginal cells (Singh et al., 2008), and third, K^{+} induced stimulation of K^{+} secretion (Wangemann et al., 1995; Wangemann et al., 1996). ROS-induced loss of KCNJ10 would abolish the endocochlear potential and hearing and the associated reduction in K^{+} flux toward marginal cells would limit the rate of K^{+} secretion, metabolism and ROS production. The reduced ROS production would then permit restoration of KCNJ10 expression, KCNJ10 channel function would restore the endocochlear potential and restore hearing but also supply increased amounts of K^{+} to marginal cells, which again would stimulate K^{+} secretion, metabolism and ROS production. Irreversible hearing loss would result when endolymphatic Ca^{2+} concentrations rise and hair cells succumb to Ca^{2+} overload (Everett et al., 2001; Wangemann et al., 2007).

4. Conclusions

Enlargement of the vestibular aqueduct (EVA) is a comparatively common but enigmatic sensorineural hearing loss disorder in children. Studies in mouse models demonstrate that enlargement and acidification of the scala media are early events in the pathogenesis of hearing loss. Future work to elucidate the mechanism of hearing loss should focus on fluid transport in cochlear development and alterations of cellular and molecular function and signaling in the lateral wall of the cochlea. The results of these studies may lead to treatment strategies to preserve hearing in humans with mutations of *SLC26A4*.

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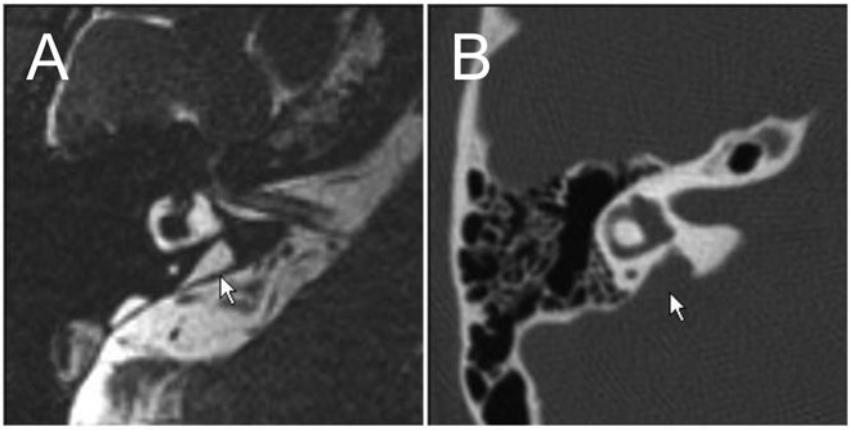

Fig 1.

Radiologic imaging of an enlarged vestibular aqueduct. A) Axial computed tomography (CT) scan of an enlarged vestibular aqueduct (arrow). B) Axial MR (magnetic resonance) image of the soft tissue correlate of an enlarged vestibular aqueduct: an enlarged endolymphatic duct and sac (arrow). Reproduced from http://www.nidcd.nih.gov/health/hearing/eva-intro.htm.

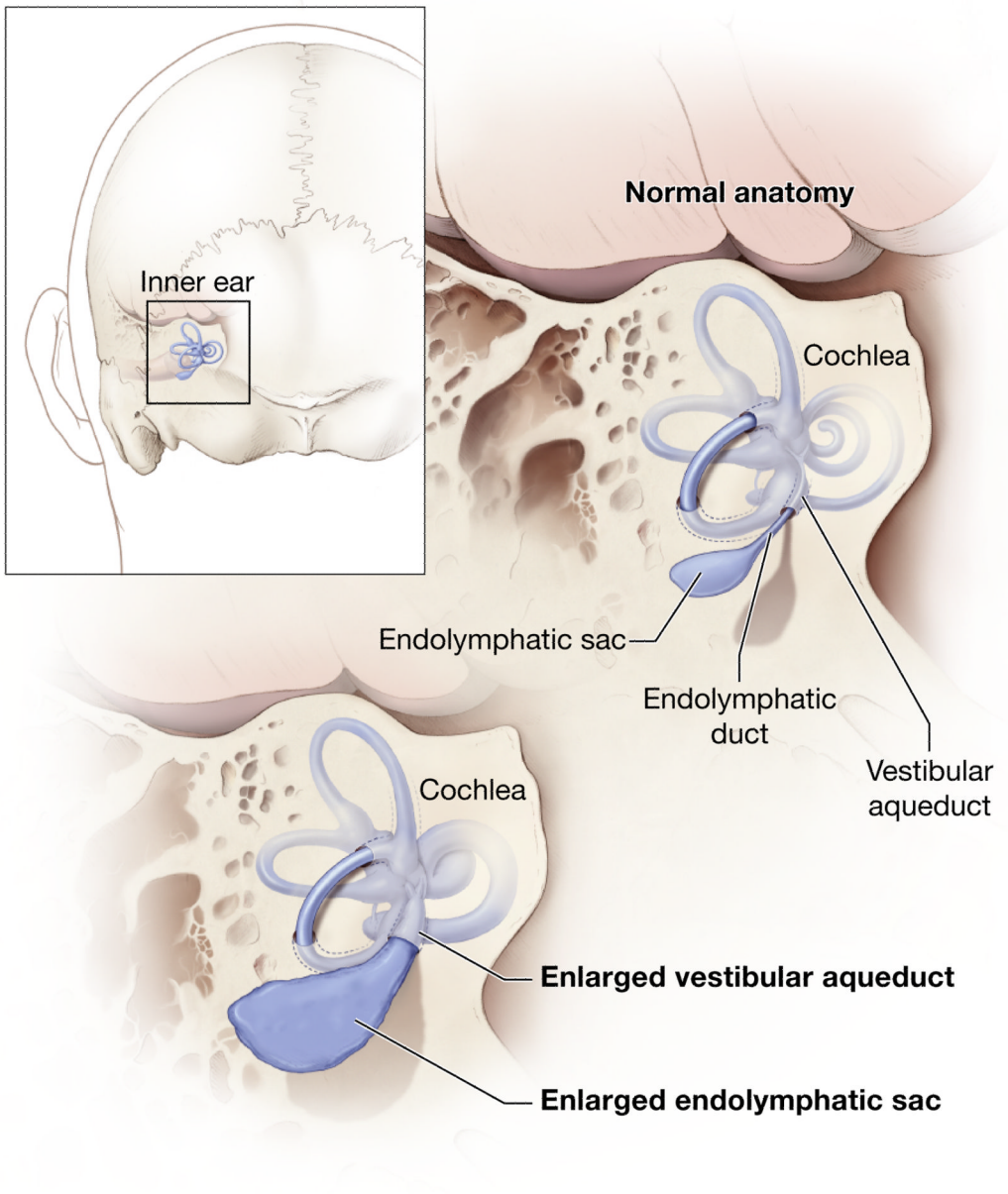

Fig 2.
Schematic illustration of an enlarged vestibular aqueduct and endolymphatic sac and duct. Reproduced from http://www.nidcd.nih.gov/health/hearing/vestAque.htm.

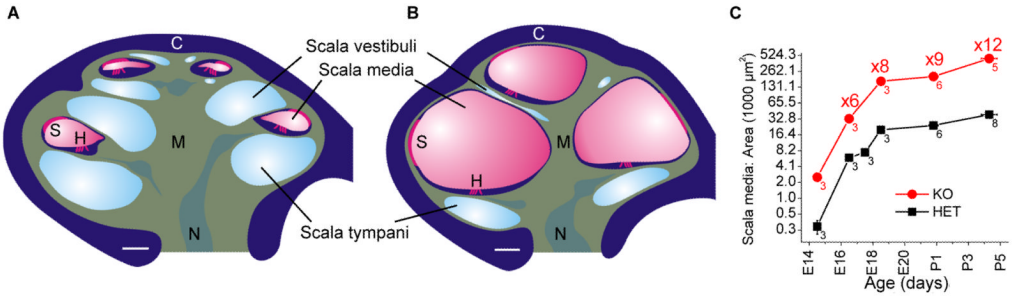

Fig 3.

Cochlear enlargement. Reproduced from Kim et al. (Kim et al., 2010). A) Diagram based on a cochlea obtained from an E18.5 *Slc26a4*$^{+/-}$ mouse. B) Diagram based on the enlarged cochlea obtained from an E18.5 *Slc26a4*$^{-/-}$ mouse. C) Measurements of cross-sectional areas of scala media from the basal turn of the cochlea in *Slc26a4*$^{+/-}$ and *Slc26a4*$^{-/-}$ mice. Note that the growth of the lumen is parallel between *Slc26a4*$^{+/-}$ and *Slc26a4*$^{-/-}$ mice and that a ~10-fold enlargement is maintained throughout development. Abbreviations: C, otic capsule; S, stria vascularis; H, sensory hair cells; M, modiolus; N, cochlear nerve. Spaces occupied by mesenchymal cell (green) are compressed in *Slc26a4*$^{-/-}$ mice, and fibrocytes in the modiolus (M) and between the otic capsule (C) and stria vascularis (S) are displaced.

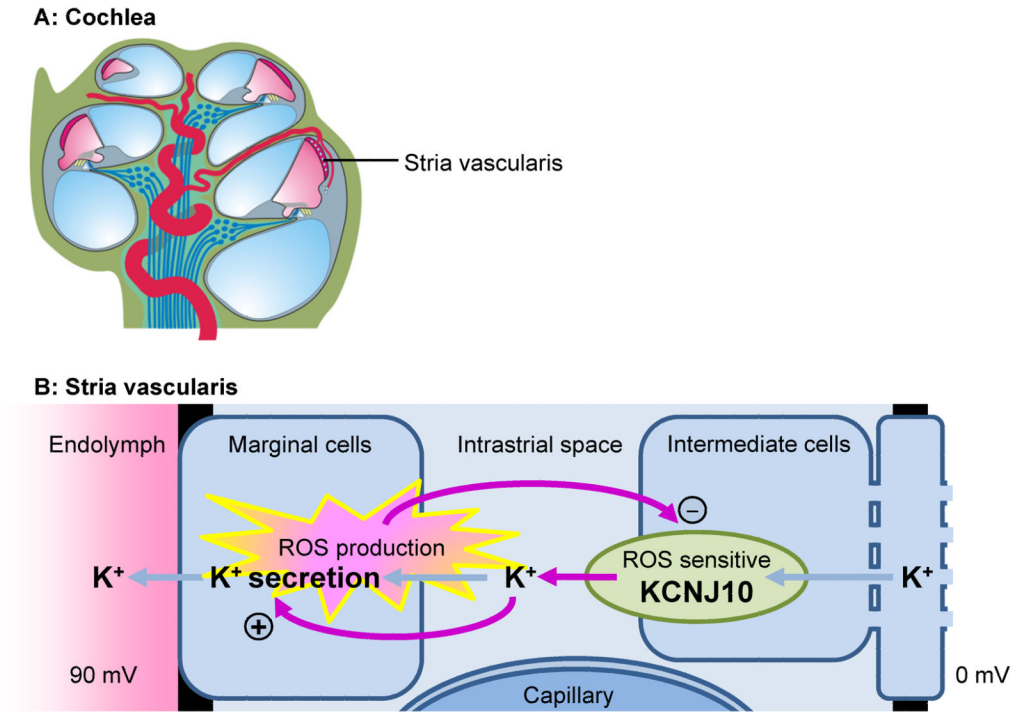

Fig 4.

Hypothetical mechanism for fluctuating hearing loss. A) Diagram based on a cochlea obtained from a P7 *Slc26a4*$^{+/-}$ mouse. B) Diagram of the stria vascularis illustrating a negative feedback mechanism that leads to fluctuating loss of KCNJ10, the K^+ channel that generates the endocochlear potential. Fluctuating loss of the endocochlear potential can be expected to the lead to fluctuating loss of hearing.