Mouse Models Reveal the Role of Pendrin in the Inner Ear

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Abstract

In 1896, Vaughan Pendred, MD, wrote a case report about two siblings that presented with hearing loss and goiter. This initial report was followed over the next 100 years with additional cases, and the condition became known as Pendred syndrome. The underlying gene, *SLC26A4*, which codes for the protein pendrin, was discovered in 1997, and mutations of *SLC26A4* have since been recognized to underlie not only Pendred syndrome but also nonsyndromic hearing loss associated with an enlargement of the vestibular aqueduct (EVA) and variable deficits in vestibular function. In 2001, Dr. Lorraine Everett, in a team led by Dr. Eric Green, reported the first mouse model that recapitulates EVA, deafness, and vestibular dysfunction. This and other mouse models have proven to be tremendously valuable in the quest to understand the role of pendrin in hearing and vestibular function. This chapter summarizes work on these mouse models that are revealing the role of pendrin in the inner ear.

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2.1 Introduction[1]

The gene *SLC26A4* (MIM #605646) codes for the protein pendrin, which is an electroneutral exchanger for anions such as HCO_3^-, Cl^-, and I^- (Scott et al. 1999; Scott and Karniski 2000; Soleimani et al. 2001). Pendrin is expressed in the apical membrane of epithelial cells in the inner ear, the thyroid, and the kidney (Royaux et al. 2000, 2001; Wangemann et al. 2004). Additional expression has been found in airways, mammary glands, uterus, placenta, liver, platelets, and adrenal glands (Bidart et al. 2000; Suzuki et al. 2002; Rillema and Hill 2003; Nakao et al. 2008; Alesutan et al. 2011; Pelzl et al. 2013; Lazo-Fernandez et al. 2015). The expression of *SLC26A4* in the inner ear and the thyroid is consistent with the observations that mutations of *SLC26A4* cause hearing loss associated with an enlarged vestibular aqueduct (EVA; MIM #600791), Mondini-like dysplasia of the cochlea, vestibular dysfunction, and enlargement of the thyroid (Pendred syndrome; MIM #274600). The prevalence and spectra of *SLC26A4* mutations vary among different populations (Park et al. 2003; Tsukada et al. 2015). The highest prevalence of *SLC26A4* mutations has been reported in East Asian populations in China, Taiwan, Japan, and Korea. Notably, in some Chinese populations, 13.7, 16.8, or 18.6 % of children attending schools for the deaf carry mutations of *SLC26A4* (Yuan et al. 2009; Zhu et al. 2015; Jiang et al. 2015). Between 82 and 98 % of East Asian patients with EVA carry mutations of *SLC26A4* (Choi et al. 2009; Tsukada et al. 2015). The prevalence of *SLC26A4* mutations in European and North American Caucasians is much lower. About 50 % of North American and European Caucasian patients with EVA carry mutations of *SLC26A4* (Campbell et al. 2001). Of these patients, about one-half carry one detectable mutation in the coding regions and splice sites of *SLC26A4*, whereas the other one-half of patients carry mutations on both alleles. Hearing loss is sometimes congenital, but typically fluctuating and progressive with an onset before or around the time of oral-auditory speech and language acquisition (Pryor et al. 2005; Choi et al. 2009; Miyagawa et al. 2014). Vestibular dysfunction is less overt and less common (Abe et al. 1999; Sugiura et al. 2005; Suzuki et al. 2007; Zhou and Gopen 2011; Zalewski et al. 2015; Jung et al. 2016), and the onset of goiter occurs typically after puberty (Fraser et al. 1960; Fraser 1965; Ajij et al. 2016). In contrast to mutations of other genes that cause hearing loss, there is no close correlation between mutation type and phenotype for *SLC26A4* (Choi et al. 2009; Tsukada et al. 2015). However, in north American and European Caucasian EVA patients, the number of mutant alleles of *SLC26A4* is correlated with both the thyroid and auditory phenotypes. Bilateral EVA and a biochemical defect in thyroid iodine organification are penetrant features of two mutant alleles of *SLC26A4*, whereas unilateral EVA and a normal thyroid iodination phenotype are observed in association with one mutant allele of *SLC26A4*. The incomplete penetrance of EVA and variability in the severity of hearing loss suggest that additional genetic or environmental factors play a

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critical role, which merits further investigations into the physiology and pathobiology of pendrin-related deafness with the goal to ameliorate or prevent hearing loss in individuals carrying mutations of *SLC26A4*.

Most research into the physiology and pathobiology of pendrin-associated hearing loss has been carried out in mice. The goal of this review is to provide a brief summary of studies in mouse models that have been developed to delineate the role of pendrin in the physiology of hearing and balance with the ultimate goal to develop strategies to preserve hearing in individuals that carry mutations of *SLC26A4*. Clinical phenotypes and the genetics of hearing loss associated with EVA are reviewed elsewhere (Griffith and Wangemann 2011; Ito et al. 2011, 2013).

2.2 Synopsis of the Development of the Murine Inner Ear

The development of the inner ear begins in mice at embryonic day 9.5 (E9.5) with the formation of an otocyst (*reviewed in*: (Mansour and Schoenwolf 2005)). The otocyst encloses amniotic fluid, which is a plasma-like fluid containing ~140 mM Na^+ and ~10 mM K^+ (Cheung and Brace 2005). Between E10 and 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, the center of the otocyst reorganizes into the vestibular labyrinth. The lumen of the endolymphatic sac opens at E10.5, and the lumen of the cochlea opens at E14.5 (Kim and Wangemann 2010). Lumen formation during the growth phase of the inner ear is controlled by a balance of fluid secretion and absorption. Fluid secretion appears to occur in the vestibular labyrinth and fluid absorption in the endolymphatic sac (Kim and Wangemann 2010). The hypothesis that NaCl secretion and NaCl absorption control luminal volume during the growth phase of the inner ear is consistent with the finding that the luminal fluid in the cochlea is filled until E17.5 with a solution that contains ~140 mM Na^+, ~126 mM Cl^-, ~10 mM K^+, and ~25 mM HCO_3^- (Kim and Wangemann 2011, Li et al. 2013b).

At E17.5, closure of the utricular-saccular duct separates the interconnected fluid compartments of the inner ear into two fluid systems (Cantos et al. 2000). One system consists of the cochlea, the saccule, and the endolymphatic sac, and the other consists of the utricle, the three *ampullae*, and the three semicircular canals (Fig. 2.1). The separation of the vestibular labyrinth from the cochlea coincides with the acquisition of mechanosensitivity of vestibular hair cells at E17 (Geleoc and Holt 2003; Lelli et al. 2009). The onset of vestibular function occurs ~8 days later, at postnatal day 4 (P4), concurrent with the general maturation of the organ and the maturation of the innervation (Nordemar 1983; Desmadryl and Sans 1990). With the conclusion of the growth phase of the inner ear, the luminal fluid, endolymph, in the cochlea and the vestibular labyrinth changes from a NaCl-rich to a KCl-rich solution. The onset of K^+ secretion in the utricle is currently under investigation. The onset of K^+ secretion in the cochlea, however, has been found to occur at ~ E19.5. Two days later, at P0, when cochlear sensory cells

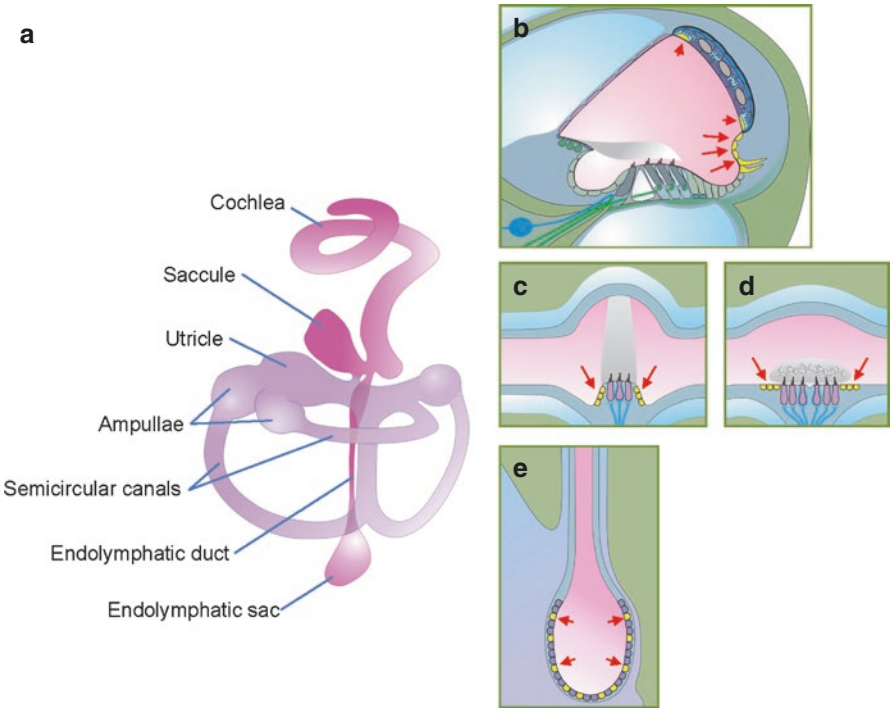

Fig. 2.1 Pendrin expression in the inner ear. (**a**) Schematic overview. The inner ear consists of the cochlea, saccule, and utricle and three *ampullae* with semicircular canals and the endolymphatic sac and duct. All compartments are lined with epithelial cells and filled with endolymph. (**b**) Cross section of one turn of the mature cochlea. Pendrin is expressed in epithelial cells of the spiral prominence (*long arrows*) and in spindle cells of *stria vascularis* (*short arrows*). (**c**–**d**) Cross sections of the saccule or utricle and an *ampulla*. Pendrin is expressed in transitional cells, which are epithelial cells surrounding the *maculae* in the saccule and utricle and the *cupulae* in the *ampullae*. (**e**) Cross sections of the endolymphatic duct and sac. The endolymphatic duct runs through a bony canal, which is called the vestibular aqueduct. Pendrin is expressed in mitochondria-rich cells in the endolymphatic sac (*arrows*). Similar diagrams were contributed to other papers (Li et al. 2013a, Wangemann 2013)

acquire mature mechanosensitivity, cochlear endolymph contains ~70 mM K^+ (Lelli et al. 2009, Li et al. 2013b). Five days later, at P3, endolymph contains ~100 mM K^+, which is close to the mature concentration of 150 mM K^+ (Li et al. 2013b). Concurrent with the rise of the K^+ concentration occurs a decline of the Na^+ concentration (Li et al. 2013b). The onset of hearing at ~P12 coincides with the maturation of the endocochlear potential, which rises between P5 and P15 from ~10 mV to the mature voltage of ~90 mV (Steel and Barkway 1989; Yamasaki et al. 2000; Wangemann et al. 2007). In the mature inner ear, the Na^+ concentration is ~1 mM and the Ca^{2+} concentration is 22 μM, both concentrations being unusually low for an extracellular fluid since most extracellular fluids contain ~150 mM Na^+ and 1.5 mM Ca^{2+}(*reviewed in*: Wangemann 2006).

2.3 Pendrin Expression in the Inner Ear

Pendrin in the inner ear functions as an exchanger of Cl^- and HCO_3^- and thereby may contribute to an elevated endolymphatic HCO_3^- concentration and an elevated endolymphatic pH (Nakaya et al. 2007; Wangemann et al. 2007; Kim and Wangemann 2011). In the mature inner ear, pendrin is expressed in the apical membrane of nonsensory epithelial cells in the cochlea, the vestibular labyrinth, and the endolymphatic sac (Royaux et al. 2003; Wangemann et al. 2004). During development, the earliest expression of pendrin in the inner ear occurs at E11.5 in the endolymphatic sac (Kim and Wangemann 2011). Between E13.5 and E14.5, expression in the endolymphatic sac increases dramatically. At E14.5, virtually all pendrin expression in the inner ear is located in the endolymphatic sac. In the cochlea, the earliest expression of pendrin is found in the hook region at E14.5. The hook region is the most basal part of the cochlea. Between E14.5 and E17.5, pendrin expression expands from the hook region to the lower and then to the upper turn of the cochlea. The onset of pendrin expression in the vestibular labyrinth occurs at E14.5 in the utricle and at E16.5 in the *ampullae*.

2.4 Mouse Models That Lack Pendrin Expression

The first mouse model, *Slc26a4*$^{\Delta/\Delta}$ (formerly called *Slc26a4*$^{-/-}$ or *Pds*$^{-/-}$), is a knockout mouse maintained in the 129S6 strain, in which exon 8 of *Slc26a4* is replaced with a neomycin cassette (Everett et al. 2001). The replacement introduces a frame shift, which prevents the generation of a functional protein. *Slc26a4*$^{\Delta/\Delta}$ mice develop an enlarged vestibular aqueduct and a Mondini-like dysplasia of the cochlea, fail to develop hearing and display vestibular deficits. The hearing and balance phenotype of *Slc26a4*$^{\Delta/\Delta}$ mice is more severe than the phenotype in most patients with DFNB4 or Pendred syndrome, who, in many cases, are born with residual hearing that often deteriorates within the first 3 years (Kim et al. 2015).

Consistent with the recessive inheritance pattern, *Slc26a4*$^{\Delta/+}$ mice develop normal sensory systems and normal hearing and balance. *Slc26a4*$^{\Delta/\Delta}$ and *Slc26a4*$^{\Delta/+}$ mice have been used extensively to investigate the consequence of a complete lack of pendrin for the development of the inner ear. Further, *Slc26a4*$^{\Delta/\Delta}$ and *Slc26a4*$^{\Delta/+}$ mice have been used as background for transgenic mouse models that feature temporally or spatially limited pendrin expression from a transgene (Choi et al. 2011, Li et al. 2013a).

Several of the phenotypic features of *Slc26a4*$^{\Delta/\Delta}$ mice have been observed in the pendrin-mutation knock-in mouse, *Slc26a4*$^{Tm1Dontuh/Tm1Dontuh}$. *Slc26a4*$^{Tm1Dontuh/Tm1Dontuh}$ mice, which are maintained in the C57BL/6 strain, contain a splice-site mutation at exon 8 of *Slc26a4*. This mutation introduces a frame shift and a new stop-codon designed to recapitulate the human mutation c.919-2A>G, which is the most prevalent mutation in China, Taiwan, and Mongolia (Lu et al. 2011; Tsukada et al. 2015). Similar to *Slc26a4*$^{\Delta/\Delta}$ mice, *Slc26a4*$^{Tm1Dontuh/Tm1Dontuh}$ mice lack exon 8 of *Slc26a4*, which leads to a similar phenotype.

The phenotypes of *Slc26a4*$^{\Delta/\Delta}$ and *Slc26a4*$^{Tm1Dontuh/Tm1Dontuh}$ mice bear a clear resemblance to the human phenotype. In contrast, *Slc26a4*$^{Tm2Dontuh/Tm2Dontuh}$ mice, also maintained in the C57BL/6 strain, contain a single-nucleotide mutation designed to recapitulate the human mutation p. H723R, which is the most prevalent mutation in Japan and Korea (Lu et al. 2013; Tsukada et al. 2015). *Slc26a4*$^{Tm2Dontuh/Tm2Dontuh}$ mice, however, have normal hearing and balance (Lu et al. 2013). The basis for this latter observation is unknown, but illustrates the challenges in developing mouse models that closely approximate the human EVA phenotype.

2.4.1 Development of the Cochlea Without Pendrin

The first pathobiological alteration of the inner ear in *Slc26a4*$^{\Delta/\Delta}$ mice is the abnormal enlargement of the luminal volume that begins at E14.5. The enlargement coincides with cochlear lumen formation and persists throughout adulthood (Everett et al. 2001; Kim and Wangemann 2011). Formation of the enlargement during the growth phase of the inner ear appears to be the consequence of an imbalance between NaCl secretion and pendrin-dependent NaCl absorption. At E18.5, when the growth phase of the inner ear comes to an end, the enlargement amounts to a ~10-fold larger volume of scala media in the cochlea of *Slc26a4*$^{\Delta/\Delta}$ mice compared to that of *Slc26a4*$^{\Delta/+}$ mice.

The second pathobiological alteration in the inner ear in *Slc26a4*$^{\Delta/\Delta}$ mice is the acidification of cochlear endolymph that begins at E15.5 and coincides with the enlargement and the failed rise in pendrin expression at E14.5 (Kim and Wangemann 2011). Acidification of the luminal fluid also occurs in the endolymphatic sac and has been documented in the mature inner ear in the cochlea and the utricle of the vestibular labyrinth (Nakaya et al. 2007; Wangemann et al. 2007).

Luminal enlargement and acidification are the primary pathobiological alterations, which distribute the effect of pendrin deficiency from pendrin-expressing cells to the entire inner ear (Kim and Wangemann 2010). Luminal acidification alters pH-sensitive mechanisms, and luminal enlargement limits cell-to-cell communication mechanisms that rely on contact or diffusible factors transmitted via the luminal or abluminal compartment. A remarkable number of secondary consequences of the lack of pendrin expression have been observed. Impaired cell-to-cell communication may be responsible for the premature onset of connexin 26 expression in basal cells of *stria vascularis* at E18.5, for the retarded development of the layered structure of *stria vascularis* observed at P3, the retarded development of the organ of Corti between age P5 and P10 that includes a delayed opening of the tunnel, failure to express BK K^+ channels in inner hair cells, and a delayed innervation of the organ of Corti (Wangemann et al. 2009; Kim and Wangemann 2011).

One of the diffusible factors affected by the impaired cell-to-cell communication appears to be thyroid hormone (T3) that is generated from the pro-hormone thyroxine (T4) produced in the thyroid gland and delivered to the inner ear via the vasculature. In the cochlea, T3 is generated from T4 in fibrocytes located in the modiolus, the spiral limbus, and the spiral ligament. Receptors for T3, however, are located in

the organ of Corti and the cochlear capsule. Enlargement and consequently delays in thyroid hormone signaling lead to signs of a local cochlear hypothyroidism that is evident between P5 and P10 from a delayed opening of the tunnel of Corti, delayed innervation, thickening of the tectorial membrane, failure to express BK K^+ channels in inner hair cells, and a delayed ossification of the cochlear capsule (Wangemann et al. 2009; Kim and Wangemann 2011; Dror et al. 2014).

Additional consequences of the enlargement include an increase in the rate of K^+ secretion by strial marginal cells (Li et al. 2013b), an increase in the rate of Na^+ absorption by Reissner's membrane epithelial cells (Kim et al. 2014), oxidative and nitrative stress in *stria vascularis* (Singh and Wangemann 2008), loss of the K^+ channel KCNJ10 in intermediate cells of *stria vascularis* and loss of the endocochlear potential (Wangemann et al. 2004), a rise in the endolymphatic Ca^{2+} concentration (Wangemann et al. 2007), and finally degeneration of sensory cells and *stria vascularis* (Jabba et al. 2006). The increase in the rate of K^+ secretion is evident from the finding that differences in endolymph K^+ concentrations between $Slc26a4^{\Delta/\Delta}$ and $Slc26a4^{\Delta/+}$ mice never exceed a factor of 2, while the volume of endolymph differs by a factor of 10 (Kim and Wangemann 2010, Li et al. 2013b). Whether the increase in the rate of K^+ secretion is a function of the enlarged luminal volume or a function of the lower pH is not known. Marginal cells sense K^+ concentrations at the apical membrane by an unknown mechanism, and low apical K^+ concentrations lead to an increase in the rate of K^+ secretion (Wangemann et al. 1995; Wangemann et al. 1996). A pH effect on K^+ secretion is also conceivable since the K^+ channel KCNQ1/KCNE1 (formerly known as KvLQT1/minK or IsK) in the apical membrane of marginal cells is activated by extracellular acidification (Unsold et al. 2000). In spite of the elevated rate of K^+ secretion, the K^+ concentration in endolymph remains slightly lower in $Slc26a4^{\Delta/\Delta}$ compared to $Slc26a4^{\Delta/+}$ or $Slc26a4^{+/+}$ mice (Royaux et al. 2003, Li et al. 2013b). The lower K^+ concentration may be balanced by a higher endolymphatic Na^+ concentration, which, in turn, may be responsible for the observed upregulation of Na^+ reabsorption and ENaC in Reissner's membrane epithelial cells (Kim et al. 2014).

A plausible consequence of higher metabolic rates necessary to maintain higher rates of K^+ secretion is oxidative and nitrative stress that results in elevated amounts of nitrated and oxidized proteins in *stria vascularis* of $Slc26a4^{\Delta/\Delta}$ mice (Singh and Wangemann 2008). Incompletely developed defense mechanisms may contribute to the free radical load. Expression levels of the K^+ channel KCNJ10 are reduced in the combined presence of oxidative and nitrative stress (Singh and Wangemann 2008), and loss of KCNJ10 is sufficient to abolish the endocochlear potential (Marcus et al. 2002). The endocochlear potential, however, depends not only on the expression of KCNJ10 but also on the integrity of epithelial and endothelial barriers that separate the intrastrial fluid space from endolymph, perilymph, and blood. Studies which used fluorescent beads (~20 nm diameter) as tracer have failed to demonstrate a compromise of the barrier between the intrastrial fluid space and blood; however, studies that used the smaller tracer biotin have demonstrated a compromise in the barrier between the intrastrial fluid space and perilymph in $Slc26a4^{\Delta/\Delta}$ mice (Ito et al. 2014). This compromise in the barrier integrity in conjunction with the loss of KCNJ10 appears to cause the observed loss of the endocochlear potential.

The loss of the endocochlear potential and the acidification of endolymph are likely the main factors that contribute to the elevation of the endolymphatic Ca^{2+} concentration in *Slc26a4*$^{\Delta/\Delta}$ mice. The concentration of Ca^{2+} in endolymph of normal mice, such as *Slc26a4*$^{\Delta/+}$ mice, is 22 μM, whereas the Ca^{2+} concentration in *Slc26a4*$^{\Delta/\Delta}$ mice is higher by a factor of ~100 (Wangemann et al. 2007). The reduction of the endocochlear potential could contribute to the observed elevation of the endolymphatic Ca^{2+} concentration since suppression of the endocochlear potential has been shown to coincide with a rise in the endolymphatic Ca^{2+} concentration (Ninoyu and Meyer zum Gottesberge 1986; Ikeda et al. 1987). The elevated luminal Ca^{2+} concentration in *Slc26a4*$^{\Delta/\Delta}$ mice is likely to contribute to the degeneration of sensory hair cells that becomes overt at P15 (Everett et al. 2001). Contributing factors may be the luminal acidification, local cochlear hypothyroidism, and the lack of the endocochlear potential. Similarly, marginal cells of *stria vascularis* degenerate after P15, and macrophages appear in stria vascularis of *Slc26a4*$^{\Delta/\Delta}$ mice at P30 (Jabba et al. 2006). Macrophages that accumulate in *stria vascularis* are strongly pigmented and give *stria vascularis* a dark appearance (Wangemann et al. 2004). It is unclear whether pigmentation is inherent to macrophages or whether pigmentation is acquired by phagocytosis of melanin-containing intermediate cells of *stria vascularis*.

2.4.2 Development of the Vestibular Labyrinth Without Pendrin

Secondary consequences of the lack of pendrin expression in the vestibular labyrinth include an increase in the endolymphatic Ca^{2+} concentration and the formation of giant otoconia which leads to vestibular dysfunction (Everett et al. 2001; Wangemann et al. 2004; Nakaya et al. 2007). Otoconia are protein-containing $CaCO_3$ crystals that enable the detection of linear acceleration in the utricle and saccule of the vestibular labyrinth. Normal otoconia vary in size but do not exceed ~20 μm, whereas giant otoconia reach sizes of ~200 μm. Giant otoconia have been reported in *Slc26a4*$^{\Delta/\Delta}$ mice as well as in *Slc26a4*$^{loop/loop}$ and *Slc26a4*$^{Tm1Dontuh/Tm1Dontuh}$ mice (Everett et al. 2001; Wangemann et al. 2004; Dror et al. 2010; Lu et al. 2011). The concentration of Ca^{2+} in vestibular endolymph of normal mice, such as *Slc26a4*$^{\Delta/+}$ mice, is 250 μM, whereas the Ca^{2+} concentration in *Slc26a4*$^{\Delta/\Delta}$ mice is higher by a factor of ~10 (Nakaya et al. 2007). The higher endolymphatic Ca^{2+} concentration may be a function of the larger transepithelial potential (−4 mV in *Slc26a4*$^{\Delta/\Delta}$ mice vs. −1.5 mV in *Slc26a4*$^{\Delta/+}$ mice), the more acidic pH (pH 7.1 in *Slc26a4*$^{\Delta/\Delta}$ mice vs. pH 7.4 in *Slc26a4*$^{\Delta/+}$ mice), and a failure to absorb Ca^{2+} (Nakaya et al. 2007; Yamauchi et al. 2010).

2.5 Mouse Models That Express Hypomorphic Pendrin

Slc26a4$^{loop/loop}$ mice were identified in a mutagenesis screen for neurosensory disorders and found to contain a point mutation, S408F, that reduces the anion exchange activity of pendrin without affecting protein expression (Dror et al. 2010). Interestingly, this hypomorphic mutant results in a phenotype that is similar to the phenotype of

Slc26a4$^{\Delta/\Delta}$ mice, which features a complete loss of pendrin expression. Similar to *Slc26a4*$^{\Delta/\Delta}$ mice, *Slc26a4*$^{loop/loop}$ mice do not acquire hearing or vestibular function, develop an enlargement of the cochlea, and form giant otoconia in the vestibular labyrinth. Further, *Slc26a4*$^{loop/loop}$ mice, similar to *Slc26a4*$^{\Delta/\Delta}$ mice, develop signs of cochlear hypothyroidism including a thickened tectorial membrane with reduced beta-tectorin expression, a lack of BK K^+ channels in inner hair cells, and a delayed ossification of the temporal bone (Dror et al. 2014). These findings underscore the importance of sufficient pendrin function during development of the inner ear.

2.6 Mouse Models with Spatially Limited Pendrin Expression

Slc26a4 transcription is controlled by various regulatory elements in the promoter region of *Slc26a4* that allow the expression to be cell-specific and responsive to specific situations (Yang et al. 2007; Adler et al. 2008; Rozenfeld et al. 2012; Vanoni et al. 2013). Notably, expression in mitochondria-rich cells of the endolymphatic sac of the inner ear is driven by the transcription factor FOXI1, whereas expression in the cochlea and the vestibular labyrinth is driven by other regulators (Yang et al. 2007). FOXI1 in mitochondria-rich cells of the endolymphatic sac drives not only the expression of pendrin but also the expression of ATP6V1B1, a subunit of the H^+ ATPase that is expressed in the inner ear only in these cells (Vidarsson et al. 2009). FOXI1 and ATP6V1B1 have therefore been used to generate mouse models that exclude the expression of pendrin from the endolymphatic sac or that limit the expression of pendrin in the inner ear to the endolymphatic sac.

2.6.1 Inner Ears Without Pendrin Expression in the Endolymphatic Sac

The mouse model *Foxi1*$^{-/-}$, which lacks expression of the transcription factor FOXI1, expresses pendrin in the cochlea and in the vestibular labyrinth but lacks pendrin expression in the endolymphatic sac (Hulander et al. 2003). The observations that *Foxi1*$^{-/-}$ mice are deaf, have vestibular dysfunction, and develop an enlargement of the inner ear point to the importance of the endolymphatic sac for the development of the cochlea and the vestibular labyrinth.

2.6.2 Inner Ear Without Pendrin Expression in the Cochlea and Vestibular Labyrinth

Slc26a4$^{\Delta/\Delta}$ and *Slc26a4*$^{\Delta/+}$ mice have been used as genetic background for a model that features a spatially limited pendrin expression (Li et al. 2013a). Tg(*B1-hPDS*)*Slc26a4*$^{\Delta/\Delta}$ mice contain a transgene, which consists of the promoter for human *ATP6V1B1* that drives the expression of human *SLC26A4*,

formerly called *hPDS*. *ATP6V1B1* codes for the B1-subunit of the H^+ ATPase, which is expressed in mitochondria-rich cells of the endolymphatic sac but not in the cochlea or in the vestibular labyrinth. Thus, Tg(*B1-hPDS*)*Slc26a4*$^{\Delta/\Delta}$ mice express human pendrin in mitochondria-rich cells of the endolymphatic sac but lack pendrin expression in the cochlea and the vestibular labyrinth of the inner ear (Li et al. 2013a). Tg(*B1-hPDS*)*Slc26a4*$^{\Delta/\Delta}$ mice do not develop the enlargement typically observed in *Slc26a4*$^{\Delta/\Delta}$ mice. This finding demonstrates that restoration of pendrin expression in the endolymphatic sac is sufficient to restore fluid absorption in the endolymphatic sac and to restore normal fluid homeostasis throughout the inner ear. Most interestingly, hearing and balance are restored in Tg(*B1-hPDS*)*Slc26a4*$^{\Delta/\Delta}$ mice. These findings raise the possibility that a spatially limited therapy focused on the endolymphatic sac (a structure that is relatively remote from the cochlea) might restore normal hearing and balance. Although this finding suggests that the expression of pendrin in the cochlea and the vestibular labyrinth is dispensable for hearing and balance, it remains to be determined whether a cochlea without pendrin has a robust hearing phenotype or whether pendrin expression has a protective or homeostatic role in response to common stressors such as noise and aging.

2.7 Mouse Models with Temporally Limited Pendrin Expression

Slc26a4$^{\Delta/\Delta}$ and *Slc26a4*$^{\Delta/+}$ mice have also been used in combination with a binary transgenic line that permits temporally limited pendrin expression. Tg[E],Tg[R]*Slc26a4*$^{\Delta/\Delta}$ mice contain two transgenes, an effector transgene, Tg[E], which consists of the murine promoter of *Slc26a4* in series with the coding sequence of the tet-on transactivator rtTA, and a responder transgene, Tg[R], which consists of a response-element for the doxycycline-bound rtTA-regulating expression of a murine *Slc26a4* cDNA (Choi et al. 2011). Since rtTA expression is driven by an *Slc26a4* promoter and regulatory elements, the temporal and spatial domains of expression of pendrin are limited to those in which pendrin is expressed in wild-type mice. Both transgenes, which were randomly inserted at unlinked locations in the genome, were crossed with the *Slc26a4*$^{\Delta/\Delta}$ line so that pendrin is expressed in the inner ear when doxycycline is present. Omission of doxycycline prohibits expression, and withdrawal of doxycycline leads to a rapid cessation of pendrin expression. Thus, pendrin expression can be controlled through the administration of doxycycline. When doxycycline was present from conception onward, mice developed a normal anatomy of the inner ear with no evidence of enlargement and normal hearing thresholds similar to *Slc26a4*$^{\Delta/+}$ mice, and when doxycycline was absent, mice developed an enlargement of the inner ear and failed to acquire hearing similar to *Slc26a4*$^{\Delta/\Delta}$ mice. Interestingly, cessation of pendrin expression in a fully functional inner ear did not affect hearing (Choi et al. 2011). These findings demonstrate that pendrin is required for the development but not for the maintenance of hearing in a normally developed ear.

2.7.1 Pendrin Expression Is Required During a Critical Time Period During Development

The mouse model Tg[E],Tg[R]*Slc26a4*$^{\Delta/\Delta}$ was used to determine the time period during which pendrin expression is required for normal cochlear development (Choi et al. 2011). Mice developed normal hearing, when doxycycline was administered from conception onward. Normal hearing thresholds developed even when the normal onset of pendrin expression at E11.5 was delayed by 5 days to E16.5 in the conditional mutant mice. Further, mice developed normal hearing thresholds, when pendrin expression was terminated by withdrawal of doxycycline as early as P2. These data define E16.5 to P2 as the most critical time period during which pendrin is needed for the development of normal hearing thresholds (Choi et al. 2011). The time period needed for the development of an uncompromised endocochlear potential and a normal endolymphatic pH begin earlier and last longer, which may imply that the hearing phenotype may be less robust in spite of normal thresholds when pendrin expression is restricted to the most critical time period (Choi et al. 2011). Taken together, the observations open the prospect that a temporally limited therapy focused on the prenatal phase of development may restore hearing in individuals bearing mutations of *SLC26A4*.

2.7.2 Pendrin Deficiency During Development Leads to Degeneration of *Stria Vascularis* and Causes Fluctuating and Progressive Hearing Loss

The mouse model Tg[E],Tg[R]*Slc26a4*$^{\Delta/\Delta}$ was used to determine the effect of pendrin deficiency on hearing thresholds and on the stability of the hearing phenotype (Ito et al. 2014). Doxycycline was administered from conception until E17.5 and thus, pendrin expression was terminated within the critical time period. Mice developed a normal anatomy of the inner ear with no evidence of an enlargement; however, the hearing phenotype was not stable. Mice developed fluctuating and progressive hearing loss, which recapitulates a hearing phenotype often observed in patients with mutations of *SLC26A4* (Griffith and Wangemann 2011; Ito et al. 2014, 2015).

Several observations point to *stria vascularis* as the major player in the development of fluctuating hearing loss (Ito et al. 2014; Ito et al. 2015). Hearing thresholds correlated with the magnitude of the endocochlear potential in pendrin-deficient mice. The expression level of KCNJ10 was reduced, and the normal complex morphological interdigitations between marginal and intermediate cells were diminished (Ito et al. 2014). Progressive hearing loss correlated with degeneration of *stria vascularis* including loss of KCNQ1 expression and enlargement of apical cell surfaces of marginal cells, hyperpigmentation, and expression of macrophage markers such as CD68 (Ito et al. 2015). Degeneration of *stria vascularis* in pendrin-deficient mice resembles, to some extent, the degeneration observed, at a faster time scale, in *Slc26a4*$^{\Delta/\Delta}$ mice that are devoid of pendrin expression (Jabba et al. 2006). The link between pendrin deficiency and degeneration of *stria vascularis* may involve the pendrin-expressing spindle-shaped cells of *stria vascularis* (Wangemann et al. 2004; Nishio et al. 2016).

Spindle-shaped cells provide the upper and lower limits of *stria vascularis*, face endolymph on their apical membrane, and connect marginal cells to basal cells (Katagiri et al. 1968; Luciano et al. 1995). Unlike marginal cells, spindle-shaped cells do not express KCNQ1 in their apical membrane but do express pendrin and ATP-gated cation channels P2RX2 (Wangemann et al. 2004; Housley et al. 2013).

2.7.3 Reinstatement of Pendrin Expression Alleviates Fluctuating Hearing Loss

The mouse model Tg[E],Tg[R]*Slc26a4*$^{\Delta/\Delta}$ was further used to determine whether restoration of pendrin expression has a beneficial effect on hearing (Nishio et al. 2016). Doxycycline was administered from conception until E17.5, which resulted in the development of fluctuating and progressive hearing loss. Doxycycline was then reinstated either at P6 or at P30, which is before or after the establishment of the endocochlear potential and the onset of hearing. Reinstatement of pendrin expression at P6 alleviated hearing fluctuations, but reinstatement at P30 had no beneficial effect. An inverse correlation was noted between hearing thresholds and the amount of pendrin expression in the apical membrane of spindle-shaped cells. These observations underscore the importance of spindle-shaped cells for the homeostasis of *stria vascularis*, link deficient spindle-shaped cells to fluctuating hearing loss, and indicate that restoration of pendrin expression may be beneficial to ameliorate progression and fluctuations of hearing in individuals bearing mutations of *SLC26A4*.

Conclusions

Studies in mouse models have provided tremendous insights into the role of pendrin in inner ear development and the etiology of an enlargement of the vestibular aqueduct. Studies of pendrin-related mouse models have revealed pathobiological mechanisms that may have broad implications beyond hearing loss associated with loss-of-function or hypo-functional mutations of *SLC26A4*. The concept that a temporally and spatially limited therapy may be sufficient to restore normal hearing or to ameliorate fluctuations and progression of hearing loss provides an imperative to develop interventions that secure a lifetime of normal hearing for individuals bearing mutations of *SLC26A4*.

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