Integration of Human and Mouse Genetics Reveals Pendrin Function in Hearing and Deafness

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Abstract
Genomic technology has completely changed the way in which we are able to diagnose human genetic mutations. Genomic techniques such as the polymerase chain reaction, linkage analysis, Sanger sequencing, and most recently, massively parallel sequencing, have allowed researchers and clinicians to identify mutations for patients with Pendred syndrome and DFNB4 non-syndromic hearing loss. While thus far most of the mutations have been in the SLC26A4 gene coding for the pendrin protein, other genetic mutations may contribute to these phenotypes as well. Furthermore, mouse models for deafness have been invaluable to help determine the mechanisms for SLC26A4-associated deafness. Further work in these areas of research will help define genotype-phenotype correlations and develop methods for therapy in the future.

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Introduction

Human mutations in *SLC26A4* lead to the most common hereditary cause of syndromic deafness known as Pendred's syndrome (PS) [1]. Several *SLC26A4* mutations have also been linked to a non-syndromic form of deafness *DFNB4*, where the ear appears to be affected exclusively. A prominent clinical characteristic of the inner ear in *SLC26A4*-related deafness is the enlarged vestibular aqueduct (EVA). In addition, PS patients show enlargement of the thyroid glands (goiter) that in some cases are associated with hypothyroidism [2]. SLC26A4, together with SLC26A3 and SLC26A6, belong to a sub-group of SLC26 proteins that function as coupled Cl^-/HCO_3^- exchangers [3]. However, SLC26A4 participates in transcellular I^- transport as well [4]. SLC26A4, also known as pendrin, is expressed in different tissues, including the inner ear [5], the luminal membrane of follicular cells in the thyroid [6], the renal cortical collecting duct [7, 8] the salivary gland ducts [4] and the respiratory tract ciliary epithelium [9]. The apparent enlargement of the thyroid gland in some of the PS individuals is a result of impaired I^- organification in the thyroid [10]. This function is probably mediated by HCO_3^-/I^- and Cl^-/I^- exchange and subsequently by active secretion of I^- into

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the thyroid follicular space. In the inner ear, the work on *Slc26a4* null mice reveals the role of pendrin in regulating the pH of endolymphatic fluids through secretion of HCO_3^- ions [11].

Functional assays have shown that different mutations within the *SLC26A4* sequence have different effects on pendrin transport activity [12]. Furthermore, whereas the number of pendrin transmembrane domains is still ambiguous in the literature [13], it is clear that some of the mutations affect pendrin sub-cellular localization so that pendrin fails to reach the plasma membrane [14]. Thus, the variable effect of each mutation on pendrin function, expression and localization can explain some of the clinical heterogeneity observed among affected individuals. Over the years, a long list of *SLC26A4* mutations related to human deafness has been published in the literature [15]. Despite the wide efforts to predict the clinical outcome of all mutations spanning the *SLC26A4* sequence, a genotype-phenotype correlation is not complete. The complex structure of pendrin as a transmembrane protein limits the ability to faithfully predict the effect of a single mutation. A resolved crystal structure of pendrin in the future may provide the possible link between specific genetic insult to the severity of the phenotype.

The aim of this review is to summarize the contribution of the cross-talk between human and mouse genetics in the study of *SLC26A4*-related deafness. By utilizing state-of-the-art sequencing technologies, the known list of pendrin mutations is expected to be enriched with novel mutations that were out of the range of conventional sequencing methods. Finally, the contribution of different mouse models enhances the ability to understand pendrin function as part of the complex network of the auditory system.

SLC26A4 (PDS) deafness mutations

Current estimates demonstrate that *SLC26A4* mutations are involved in 4% -10% of hereditary hearing loss [15, 16], with close to 200 mutations involved in both Pendred syndrome (PS; MIM#274600) and non-syndromic hearing loss (NSHL) *DFNB4* (MIM#600791) (http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm). Most mutations are associated with temporal bone abnormalities ranging from isolated enlarged vestibular aqueduct (EVA) to Mondini dysplasia [16]. The mutations are located throughout the coding region and include missense, nonsense, splice site and frameshift mutations [15]. The hearing impairment associated with mutations in *SLC26A4* is primarily sensorineural, variable in severity; bilateral or unilateral; congenital, prelingual or perilingual onset; frequently beginning at high frequencies, and sometimes has a fluctuating and/or progressive course [17]. In many cases, there is a conductive component, although the middle ear remains intact. It has been suggested that the large vestibular aqueduct functions as a third mobile window in the inner ear, resulting in an air-bone gap at low frequencies [18].

In many populations, mutations in the *SLC26A4* gene are the major genetic cause of temporal bone inner ear malformations, accounting for up to 90% of the typical PS population, 80% of individuals with EVA and the same rate among patients with Mondini dysplasia [19-21]. It is inconclusive whether the presence or absence of cochlear malformations is related to the severity of HL or whether or not there is a genotype-phenotype correlation. Pryor et al. [22] summarized that two mutant *SLC26A4* alleles are involved in all PS patients, whereas the NS EVA patients had either one or no *SLC26A4* mutations, suggesting that PS and NS EVA are distinct clinical and genetic phenomena. An additional study among French Caucasian families with NSHL and EVA [16] reported that patients with biallelic mutations had more severe deafness, an earlier age of onset, and a more fluctuating course of hearing levels than patients in whom no mutation was identified. In another study [23], a significant difference was found in the distribution of *SLC26A4* mutations in PS versus non-PS (EVA–Mondini) patients, with PS patients more likely to have two mutations comparing to one or none in non-PS. Although heterogeneity was observed in PS patients, it was lower than observed in NS EVA–Mondini. Further analysis of EVA-Mondini patients also showed that two mutations are more likely associated with Mondini dysplasia compared to one or zero associated with EVA [23].

While these studies report genotype-phenotype correlations, most studies do not find any kind of correlation with respect to hearing severity, fluctuation, progression, vertigo, or goiter, as the same combination of mutations have been described that result in variable phenotypic expression. These phenotypes range from isolated NSHL to NS EVA to Mondini dysplasia to PS, suggesting that the same etiology underlies all conditions [21, 24]. Furthermore, phenotypes are variable, even with the same mutations. T416P [25], L445W [26], H723R [21, 27] and the IVS7-2A>G [28] are involved in either PS or NSHL. Moreover, even intrafamilial phenotypic variability

was observed, e.g., the L445W mutation was identified in all affected individuals of a large family, either with PS or with NSHL [26]. Among another family with three offspring with compound R409H/1561_1571CTTGGAATGGC PS mutations, extreme variability was observed, both for the degree of impairment and the age of onset [29]. These findings are supported by localization assays, where retention of pendrin in the endoplasmic reticulum (ER) was observed, rather than targeting to the plasma membrane, for mutations involved in PS, including L236P, T416P, G384E and V239D [30, 31] as well as for mutations associated with NSHL, including T410M, Q446R and c.1458_1459insT [14, 32].

The lack of genotype-phenotype correlation and the excess of NSHL cases, with or without EVA, with only one or zero *SLC26A4* mutation suggest that NSHL/EVA/PS is a complex disease involving other genetic factors. This assumption led to the detection of digenic heterozygosity of *SLC26A4/FOXI1* and *SLC26A4/KCNJ10* mutations [33, 34]. *FOXI1*, a transcriptional regulating factor of *SLC26A4*, and *KCNJ10*, have also been implicated in the development of inner ear pathology. Interestingly, *Kcnj10* expression is downregulated in *Slc26a4*-depleted mice, which contributes to the failure of endocochlear potential generation [35]. *FOXI1* mutations were observed in PS and non-syndromic EVA patients and an additional NS EVA patient was found to be double heterozygous for *SLC26A4/FOXI1* [34]. Similarly, *KCNJ10* mutations were found in NS EVA patients as well as in a double heterozygosity state with a *SLC26A4* mutation, *SLC26A4/KCNJ10* [33]. Thus, *FOXI1* and *KCNJ10* are two genes that may contribute to the understanding of EVA heterogeneity. There are most likely many more genes and other factors to be identified, including modifier genes, or/and nutritional factors including iodine uptake [14, 21], which may determine the thyroid phenotype and the differences between and within PS and NSHL.

The high prevalence of *SLC26A4* mutations involved in hereditary HL and its involvement in disease phenotypes emphasizes the importance of the molecular characterization of the *SLC26A4* gene, as well as the identification of additional interactors/modifiers in the diagnosis of deafness. To date, routine clinical diagnostic tests for deafness have consisted of screening for the relevant mutations in a certain population. Comprehensive testing for the entire gene is not done routinely due to high costs. Implementing advanced sequencing technologies for clinical use might overcome this limitation and lead to the identification of additional mutations in a larger *SLC26A4*-associated-deafness group, enabling further genotype-phenotype analysis in larger cohorts. Recently, such an approach has been employed by applying DNA capture and massively parallel sequencing to identify inherited mutations involved in HL, including 246 genes involved in both human and mouse HL [36]. This technique also has the ability to speed up the discovery of genes that might interact and influence each other, as all variants in all genes associated with deafness may be identified in one experiment. Whole exome or genome sequencing might be an even better tool for interactors/modifiers detection, but the challenge of analyzing the data and determining which variants are the causative ones still remains.
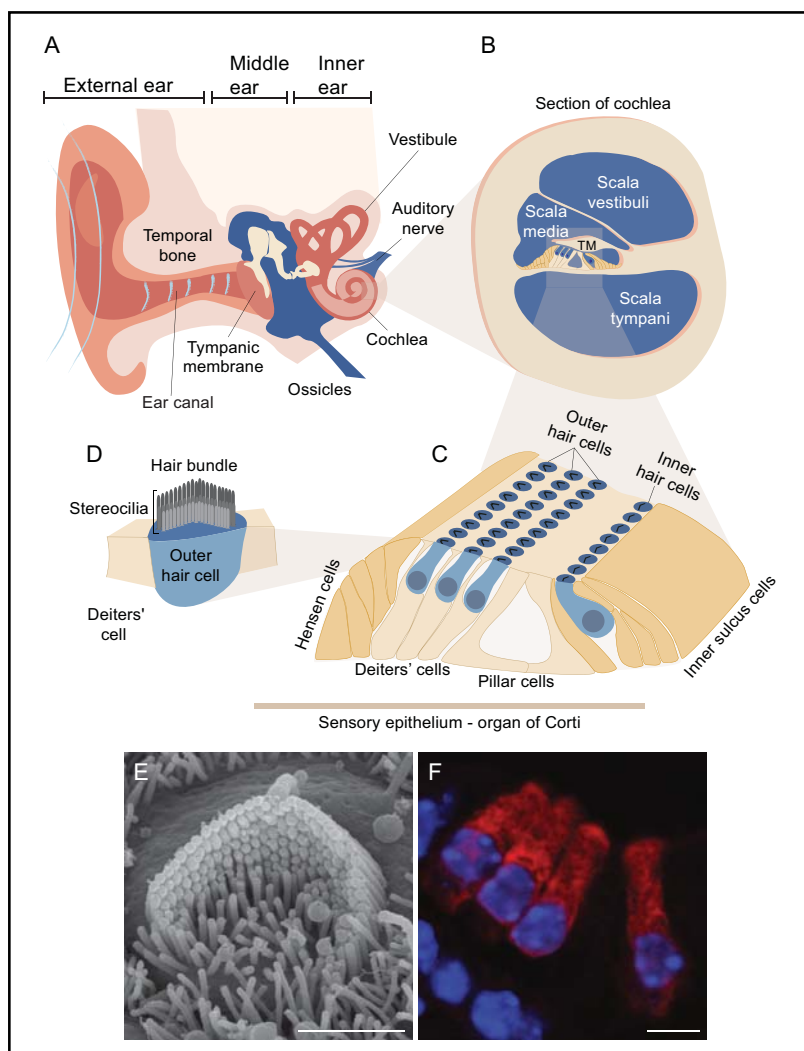
Pendrin: crosstalk between human and mouse

A wide array of organisms, including zebrafish, chick and mouse, has complemented the genetics of deafness field with an in-depth understanding of protein function. Among the models, the striking similarity between the human and mouse inner ear structure and function has defined the mouse as a prominent animal model for human deafness [37]. The tools of gene overexpression, depletion and targeted mutagenesis has enabled researchers to create reliable animal models for genetic forms of hearing loss in order to mimic the corresponding mutation in humans. Once a novel human deafness gene is discovered, the generation of an animal model is optimal for studying its function.

Two major approaches are commonly used for cloning and studying unknown genes. The reverse genetics approach (genotype-driven) begins with a candidate gene chosen by bioinformatics tools e.g. homology to other known genes. The candidate gene is 'knocked out' in a conditional/permanent manner or mutated in an animal model for further investigation [38]. The resulting knockout phenotype may be one out of the three following scenarios: compatible with the working hypothesis, an unexpected or no phenotype. In contrast, with the forward genetics approach (phenotypic-driven), no prior assumption about any of the mutated genes is made. Instead, by 'shooting in the dark,' mutations are randomly generated and the phenotype is screened. The phenotype of interest is chosen and the journey to identify the gene begins. The chemically-induced *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis is such an approach [39].

Following the positional cloning of *SLC26A4* [1], a

Fig. 1. A general anatomical structure of the auditory system is demonstrated by schematic illustration. A: The ear is divided into three distinct anatomical compartments, the outer, the middle and the inner ear. Sound waves captured by the auricle of the external ear passes through the ear canal reaching the tympanic membrane leading to its vibrations. The middle ear conducts the mechanical energy of the sound vibration and transfers it to the inner ear. B: A cross section through the auditory portion of the inner ear (cochlea) reveals its associated fluid field compartments. C: A specialized sensory organ (organ of Corti) resides within the scala media along the coil shape of the cochlea from base to apex. The inner and outer hair cells are constantly elicited by mechanical forces driven by the acoustic stimulus. D: The apical surfaces of the hair cell contain hair bundle structures assembled by actin rich stereocilia with a typical staircase structure. Upon deflection of the hair bundle, mechanoelectrical channels position at the tip of two adjacent stereocilia are open and leads to hair cell depolarization. Complex innervations at the basal pole of the sensory cells propagate the electrical signal through the auditory nerve into the brain. E: A scanning electron micrograph showing hair bundle of an outer hair cell isolated from a newborn mouse. F: A cross section through the organ of Corti shows the arrangement of the inner hair cells with respect to three rows of outer hair cells. Florescent markers were applied for myosin VI (red) and nuclear DAPI staining (blue). Scale bars equal 2 μm in panel E, 4 μm in panel. Adapted from [37] with permission.
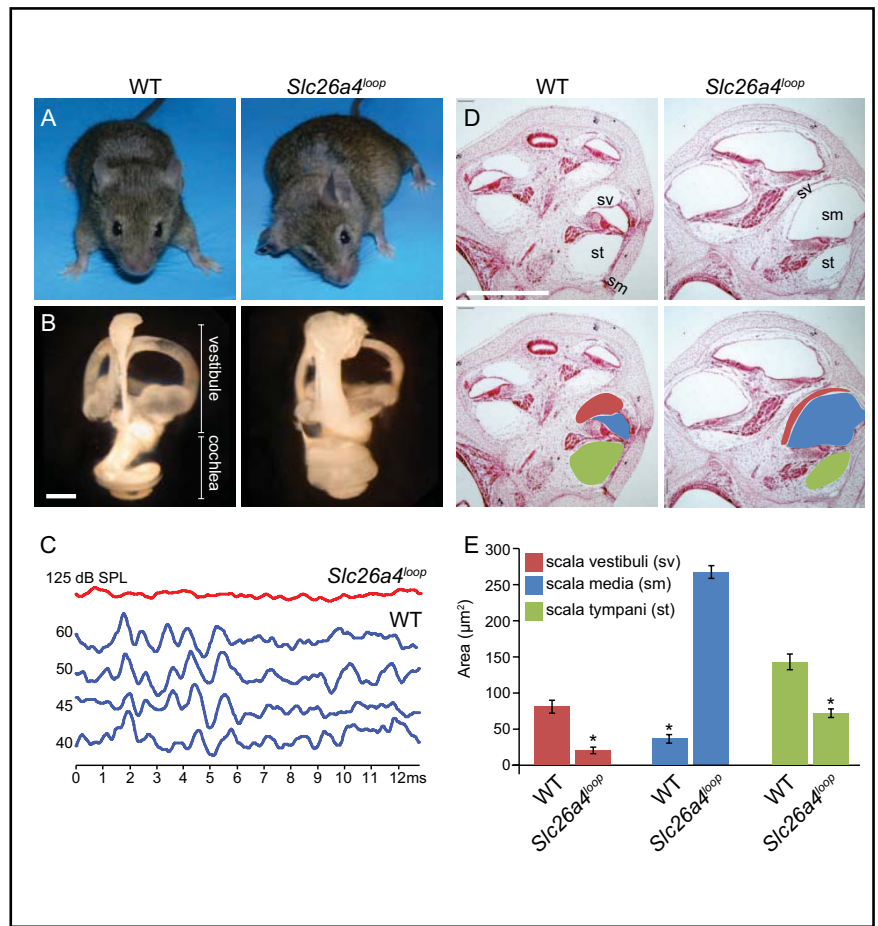

knock-out mouse model for PS was the first to be established [40]. The work on the *Slc26a4* null mice illuminated the physiological and functional role of pendrin in various systems, including the inner ear (reviewed in this issue by Wangemann et. al.). The generation of additional *Slc26a4* knockout mouse was reported in a kidney study which focused on pendrin function in the urinary system [41]. To further understand the clinical variability observed in humans carrying different mutations of pendrin, three additional mouse models were investigated. The *Slc26a4loop* mouse, generated by ENU mutagenesis, led to the discovery of new inner ear pathology that has complemented the work on the *Slc26a4* null mice with its novel phenotypic variation [42]. A spontaneous mutation in *Slc26a4* was found in the *Slc26a4pdsm* mouse model (http://mousemutant.jax.org/articles/mmrmutantpdsm.html). The reported inner ear characteristics of *Slc26a4pdsm* appear to be similar to the previously studied mouse strains. Finally, the attempt to expand the mouse mutations spectrum had led to the generation of the *Slc26a4tm1Dontuh* knock-in mouse that carries a common East Asian mutation [43]. In the following section we highlight the similarity and differences between the available *Slc26a4* mouse models, with an emphasis on the inner ear.

Common auditory dysfunction of *Slc26a4* mouse models

The inner ear is a remarkable organ that contains both the auditory and vestibular systems responsible for two prominent functions. Due to the close anatomical proximity, these two systems share many genetic, physiological and functional elements in common. As a result, many genetic mutations that affect the auditory system have consequences on the vestibular system in a direct or indirect manner. The general structure of the

Fig. 2. Auditory phenotypic characterization of *Slc26a4^loop* is common to all known *Slc26a4* mouse models. A: A representative image of *Slc26a4^loop* mice shows the typical unsteady gait of the mutant mice that was determined by a panel of vestibular behavioral tests. B: Paint-filled inner ears of *Slc26a4^loop* P0 mice show a bulged fluid filled compartment, with a prominent volume increase in the cochlea and the vestibular system. C: Auditory brainstem response (ABR) test on 8-week old mice reveals that *Slc26a4^loop* mutants are profoundly deaf at three frequencies that were tested, 8Khz, 16Khz and 32Khz. An ABR recording for 8Khz is shown (red). D: Cross sections through the cochlea of *Slc26a4^loop* mice reveals hydrops of the endolymphatic spaces of the scala media (sm), whereas the perilymphatic spaces of the scala vestibuli (sv) and scala tympani (st) are smaller. E: Histogram graphic representation shows the quantified area of the different cochlear compartments as compared between normal and mutant mice. A prominent enlargement of the endolymph field scala media is apparent in *Slc26a4^loop* mice, at the expense of the smaller scala vestibuli and scala tympani. Scale bars equal 500 µm in panel B and D. Adapted from [42] with permission.
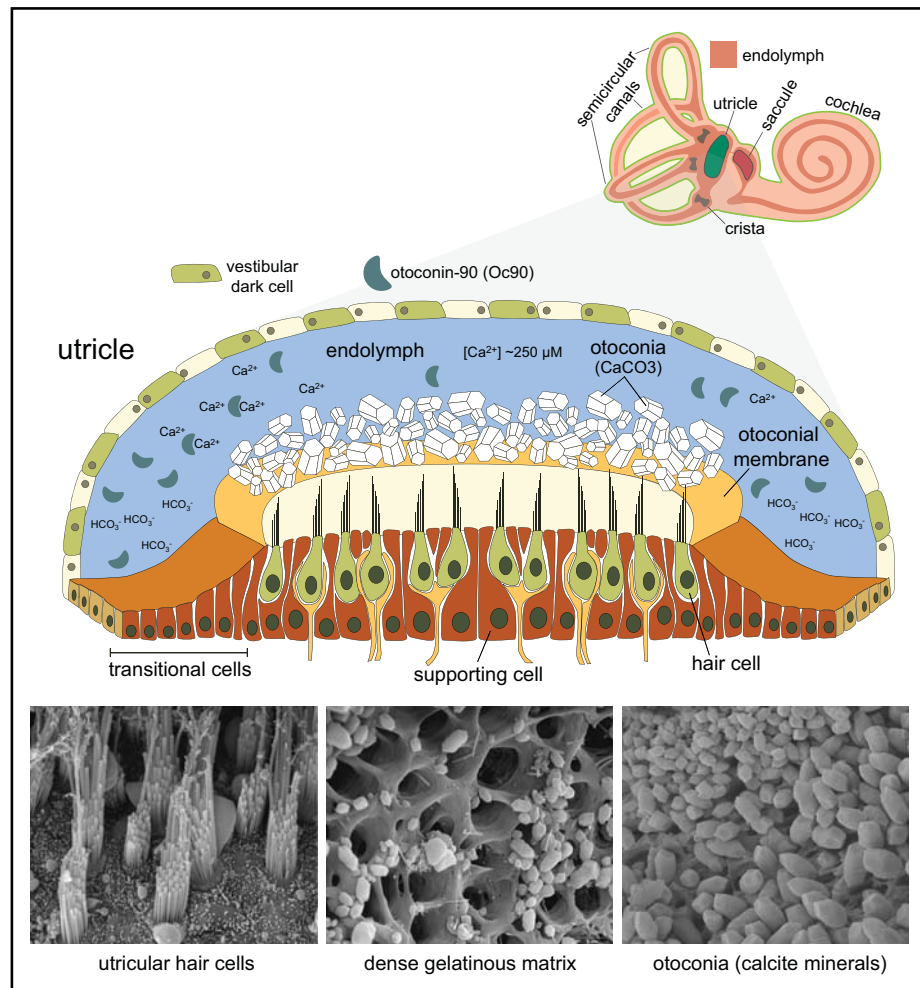

ear (Fig. 1) can be divided functionally into the conductive portion, comprised of the outer and middle ear, and the sensitive sensory portion of the inner ear (Fig. 1A). The inner ear is quite vulnerable to damage, as is evident by the long list of known human mutations that leads to hearing impairment [44]. The cochlea is a snail shape structure that contains the sensory organ of the ear known as the organ of Corti [45]. This sensory epithelium is associated with an endolymph field compartment called the scala media (Fig. 1B). The sensory cells of the auditory system, known as hair cells, are stimulated by mechanical energy originating from acoustic stimuli (Fig. 1C). Specialized actin rich protrusions at the apical suface of the cell form an organized structure of a hair bundle that is immersed in endolymph (Fig. 1D). Upon deflection of the hair bundle an influx of ions depolarize the cells, propagated via channels that are located between the tips of neighboring stereocilia [46, 47]. The unique chemical composition of the endolymph, with its high potassium (K^+) and low sodium (Na^+) and calcium (Ca^{+2}) concentrations, is essential for

hair cell depolarization and normal auditory function [48]. Hence, it should come as no surprise that many of the known genetic insults that lead to deafness affect channels and transporters that maintains the delicate homeostasis of the inner ear fluids [49]. Among this list of proteins, pendrin stands out in its prominent role of maintaining the acid-base balance of the endolymph and plays a cardinal function in the normal hearing mechanism.

Generation of pendrin null mice has provided a tremendous tool in understanding the pathophysiology underlying this form of deafness observed in humans [40]. Initial characterization of the mouse clearly demonstrated the high similarity between the clinical pathogenesis of PS patients and the mouse phenotype. The auditory brainstem response (ABR) tests show that *Slc26a4^-/-* mice are profoundly deaf, mimicking the early onset deafness in humans. Furthermore, *Slc26a4^-/-* mice show a dramatic enlargement of the endolymphatic compartment with significant hydrops of the cochlea, as well as the endolymphatic sac and duct [40]. The changes

Fig. 3. The sensory maculae of the vestibular system. Out of the five sensory organs of the vestibular system, the utricle and saccule share similar anatomical and morphological structures. Top: A cross section through the utricular sac illustrates the thick sensory epithelium creating the floor of the maculae. The hair cells (green) surrounded by supporting cells (brown) are connected into nerve fibers (yellow) in their basilar pole. The apical surface of the hair cells is composed of hair bundles of actin rich stereocilia (black) that protrude into dense gelatinous matrix. A large number of calcite crystals, known as otoconia (white), are positioned on top of the epithelium. The inertial mass of these mineralized crystals triggers the hair cells by transferring mechanical energy in response to linear movement of the body. The sac is surrounded by epithelial cells and contains endolymphatic fluids. Bottom: SEM showing the hair cells, dense gelatinous matrix and otoconia. Adapted from [42] with permission.

utricular hair cells · dense gelatinous matrix · otoconia (calcite minerals)

in inner ear fluid compartments correlates with the typical enlargements of the vestibular aqueducts (EVA) of the affected individuals, as seen by computed tomography (CT) scan [28]. Subsequent work on the *Slc26a4^loop* ([42] and Fig. 2) and the *Slc26a4^{tm1Dontuh/tm1Dontuh}* [43] mice reported similar auditory characteristics, complementing the work on the *Slc26a4^{-/-}* mice.

In the inner ear, pendrin is localized to the membrane of different cell types that face the endolymph, both in the cochlear and vestibular apparatus. Electrophysiological studies have shown that lack of pendrin leads to acidification of the endolymph, suggesting that pendrin mediates bicarbonate (HCO_3^-) secretion in the inner ear that buffers the accumulation of protons (H^+) [11]. A subsequent increase of endolymphatic Ca^{2+} ion concentration in pendrin null mice is attributed to the lower pH level that inhibits the acid-sensitive TRPV5 and TRPV6 calcium channels [50]. Pendrin null mice also failed to develop endocochlear potential and hearing due to the loss of *Kcnj10* protein expression after the age of P10 [35]. In the cochlea, *Kcnj10*, encoding a K^+ channel, is normally expressed in intermediate cells of the stria vascularis and is sufficient for generating an endocochlear potential. An observed increased level of oxidative stress in pendrin null mice impairs normal function of the stria vascularis with subsequent loss of *Kcnj10* protein expression and lack of endocochlear potential [51]. Interestingly, *KCNJ10*, together with mutations of *SLC26A4,* leads to digenic hearing loss with enlarged vestibular aqueduct [33]. Understanding that the pendrin mouse model fails to develop hearing due to the loss of the endocochlear potential defines window of opportunities for therapeutic approaches prior to the loss of KCNJ10 protein expression.

Unique vestibular dysfunction in the *Slc26a4^loop* mouse model

The vestibular system is comprised of five sensory organs (Fig. 3). Three cristae connected to semicircular canals are sensitive for angular movement and saccule and utricle are sensitive for linear acceleration and gravity [52]. The integrated input of these sensory patches is
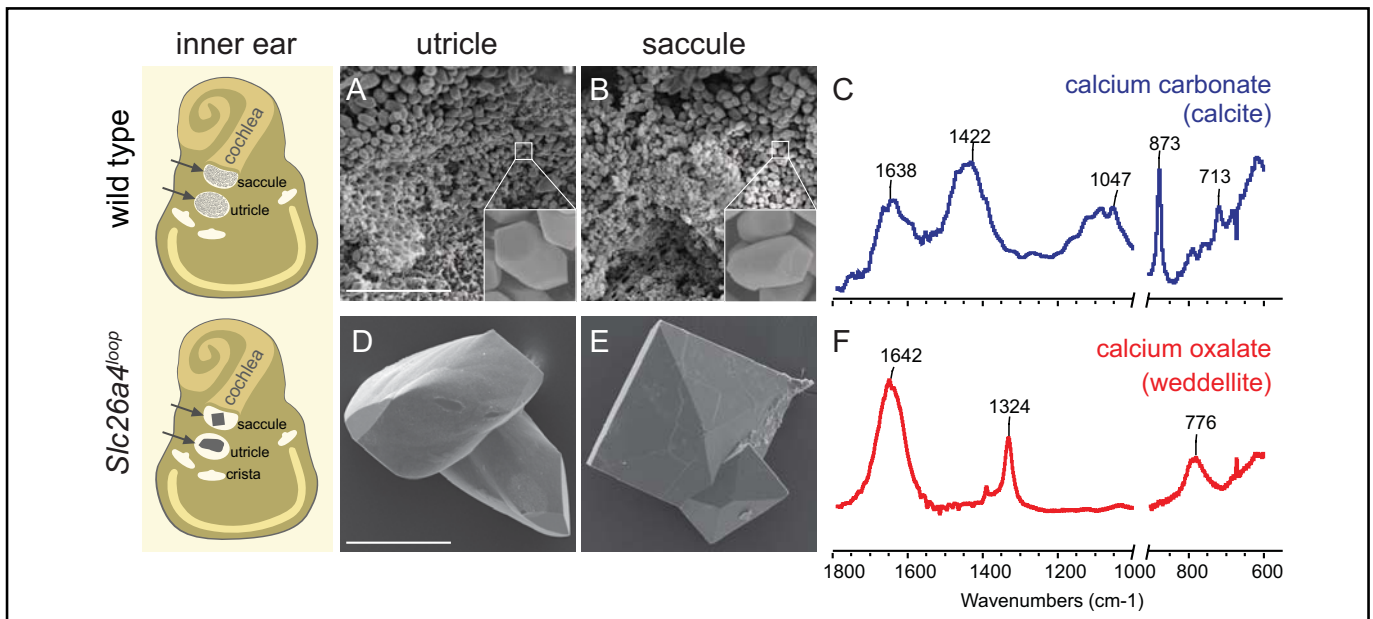
Fig. 4. Calcium oxalate minerals in the *Slc26a4^loop^* vestibular system is a unique phenotype among *Slc26a4* mouse models. Left panel: Schematic illustration of the inner ear summarizing the morphological differences of the inner ear stones at the age of 10 months old. The two sensory maculae of the vestibular system, utricle and saccule are indicated (arrows). A, B: SEM images of the utricle and saccule of a wild-type mouse shows the small (~7 µm) otoconia with typical morphology (inset in A and B). C: The spectrum of wild-type otoconia, showing the typical calcite vibrations at 713, 875 and 1422 cm^{-1}. D, E: Minerals extracted from the utricle and saccule of *Slc26a4^loop^* mutant mice. The utricle minerals are larger relative to wild-type otoconia. In the saccule, a new type of mineral can be found with crystal morphology typical of Ca-oxalate dihydrate (weddelite) crystals. F: Spectrum of the mineral extracted from the saccule in *Slc26a4^loop^* mutant mice shows vibrations at 1324 and 776 cm^{-1} that are typical of weddelite crystals. Scale bars equal 50 µm in panel A applies to B, 100 µm in panel D applies to E. Adapted from [42] with permission.

essential for three dimensional acquisition and gravity perception. Similar to the cochlea, the sensory cells of the vestibular system bathe in the (K$^+$) rich endolymph and are elicited in response to a mechanical stimulation. However, unlike the cochlea, the vestibular sensory epithelia have a different morphology and depend on a unique inertial mass that is required for its proper stimulation. The utricle and saccule are two small sacs that contain an oval-shaped thick epithelium, known as maculae, that contains the sensory hair cells. The sensory epithelia of the utricle and saccule are anatomically positioned 90° to each other [53], correlating with their functional significance in sensing linear and vertical acceleration, respectively. The apical surface of the sensory epithelium is associated with an extracellular gelatinous matrix on which large number of calcium carbonate (CaCO$_3$) crystals, also known as otoconia, are positioned. Otoconia are small highly dense calcitic minerals that associate exclusively with the saccule and utricle. Thousands of otoconia, partially embedded in a gelatinous matrix, are supported on the sensory epithelium and serve as an inertial mass that is critical for mechanical

stimulation [54, 55]. Movement of the otoconial layer through action of gravitational acceleration forces activate the underlying mechanosensory hair cells to generate action potentials that are transmitted to the brain.

In addition to the auditory deficit, all *Slc26a4* mouse models show prominent vestibular dysfunction with variable manifestations [40, 42, 43]. Among the various vestibular deficits, a prominent abnormal gait, circling behavior and abnormal reaching response clearly distinguishes the mutant mice from their littermate controls. A closer look at the utricle and saccule of these mouse models reveals an abnormal giant otoconia associated with the sensory epithelium [40]. The formation of the giant stones breaks the delicate balance between otoconia and the underlying hair cells. While in normal conditions the otoconia sustain an equally distributed mass to elicit the hair cells, in the presence of *Slc26a4* mutations a sporadic distribution of the giant minerals leads to a differential mass over the sensory cells. Whereas some hair cells are overloaded with the weight of the giant stones, others lack any viable load of otoconia minerals. As a result, both cell populations are neutralized from

being active in vestibular sensation and leads to severely disturb vestibular perception. Interestingly, out of the three *Slc26a4* mouse models, the *Slc26a4loop* mice shows a unique composition of the pathological mineralized bodies [42]. A gradual change in composition of the *Slc26a4loop* giant otoconia leads to the formation of calcium oxalate minerals, determinately different from the calcium carbonate constituent of the wild-type otoconia (Fig. 4). The changes in chemical structure of the minerals are suggested to be attributed to an ongoing progressive acidification of the surrounding fluids as a result of impaired pendrin function. A lower pH leads to dissolution of the native calcium carbonate mineral while it is in favor of calcium oxalate mineral aggregations that are more stable in acidic environment. Prominently the formation of calcium oxalate stones are most known in the kidney under pathological conditions [56]. A recent study shows that *Slc26a4* ablation leads to calcium wasting in urine due to down regulation of calcium absorbing protein in the kidney [57]. This evidence supports the leading framework that extracellular pH affects homeostasis in other organs via pH sensitive calcium channels [50]. The nucleation of oxalate crystals in *Slc26a4loop* inner ears is a unique example that links a genetic mutation to this aberrant pathophysiology [42]. Interestingly, in addition to the aberrant composition of *Slc26a4loop* giant minerals this strain distinguishes from other *Slc26a4* mouse models in the morphology of the vestibular hair cells. At early progressive ages both *Slc26a4-/-* and *Slc26a4tm1Dontuh/ tm1Dontuh* mice show massive degeneration of utricular and saccular sensory cells [40, 43], whereas in *Slc26a4loop* mouse the vestibular hair cells are intact at the same time point [42]. Although the molecular mechanism for this variation is yet to be determined, it highlights the importance of having several mouse models to illuminate the function of pendrin through different genetic mutations.

Summary

The field of genetics and its associated area of genomics have evolved tremendously over the past years. Since the discovery of *SLC26A4* mutations and association with disease, there has been remarkable progress in understanding pendrin function due to technological genomic advances. The wide expression of pendrin in a variety of systems initiated studies that illuminated the role of pendrin in different clinical conditions including deafness [11], asthma and chronic obstructive pulmonary disease (COPD) [58], kidney and chloride reabsorption [59] and iodide homeostasis of the thyroid [10]. However, despite progress, there are aspects of the role of pendrin that still remain to be explored. The absence of precise genotype-phenotype correlations, as well as modifiers of disease, of the known pendrin mutations highlight intriguing questions. The overview provided in this summary lays the groundwork for these questions and points to a promising future in the pendrin field.

Abbreviations

PS (Pendred syndrome); EVA (enlarged vestibular aqueduct); NSHL (non-syndromic hearing loss); ER (endoplasmic reticulum); EN (*N*-ethyl-*N*-nitrosourea); ABR (auditory brainstem response).

Acknowledgements

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