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Molecular and Functional Characterization of Human Pendrin and its Allelic Variants

Silvia Dossena[1], Charity Nofziger[1], Grazia Tamma[2], Emanuele Bernardinelli[1], Simone Vanoni[1], Christoph Nowak[3,4], Elisabeth Grabmayer[1], Sonja Kössler[1], Susanne Stephan[1], Wolfgang Patsch[1] and Markus Paulmichl[1]

[1]Institute of Pharmacology and Toxicology, Paracelsus Medical University, Salzburg, [2]Department of General and Environmental Physiology, University of Bari, Bari, [3]Austrian Institute of Technology, Vienna, [4]Centre of Biomimetic Sensor Science, Singapore

Key Words

Pendrin • Pendred syndrome • Deafness • Mutations • Functional test • Ion transport

Abstract

Pendrin (SLC26A4, PDS) is an electroneutral anion exchanger transporting I^-, Cl^-, HCO_3^-, OH^-, SCN^- and formate. In the thyroid, pendrin is expressed at the apical membrane of the follicular epithelium and may be involved in mediating apical iodide efflux into the follicle; in the inner ear, it plays a crucial role in the conditioning of the pH and ion composition of the endolymph; in the kidney, it may exert a role in pH homeostasis and regulation of blood pressure. Mutations of the pendrin gene can lead to syndromic and non-syndromic hearing loss with EVA (**e**nlarged **v**estibular **a**queduct). Functional tests of mutated pendrin allelic variants found in patients with Pendred syndrome or **n**on-**s**yndromic EVA (ns-EVA) revealed that the pathological phenotype is due to the reduction or loss of function of the ion transport activity. The diagnosis of Pendred syndrome and ns-EVA can be difficult because of the presence of phenocopies of Pendred syndrome and benign polymorphisms occurring in the general population. As a consequence, defining whether or not an allelic variant is pathogenic is crucial. Recently, we found that the two parameters used so far to assess the pathogenic potential of a mutation, i.e. low incidence in the control population, and substitution of evolutionary conserved amino acids, are not always reliable for predicting the functionality of pendrin allelic variants; actually, we identified mutations occurring with the same frequency in the cohort of hearing impaired patients and in the control group of normal hearing individuals. Moreover, we identified functional polymorphisms affecting highly conserved amino acids. As a general rule however, we observed a complete loss of function for all truncations and amino acid substitutions involving a proline. In this view, clinical and radiological studies should be combined with genetic and molecular studies for a definitive diagnosis. In performing genetic studies, the possibility that the mutation could affect regions other than the pendrin coding region, such as its promoter region and/or the coding regions of functionally related genes (*FOXI1, KCNJ10*), should be taken into account. The presence of benign polymorphisms in the population suggests that genetic studies should be corroborated by functional studies; in this context, the existence of hypo-functional variants and possible differences between the I^-/Cl^-

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Markus Paulmichl and Silvia Dossena,
Institute of Pharmacology and Toxicology, Paracelsus Medical University
Strubergasse 21, Salzburg, A-5020 (Austria)
Tel. +436624420021230 and +43-662-442002-1235, Fax +436624420021239
E-Mail markus.paulmichl@pmu.ac.at, E-Mail silvia.dossena@pmu.ac.at

and Cl^-/HCO_3^- exchange activities should be carefully evaluated.

Tissue expression and physiological role of pendrin

The pendrin protein was initially described as a gene product that, if mutated, is responsible for the Pendred syndrome (OMIM#274600) [1]. Human pendrin (SCL26A4, PDS) is a 780 amino acid membrane protein with transport function, expressed in tissues as diverse as the thyroid gland [2], kidney [3], inner ear [4, 5], airways [6], mammary gland [7], testis [8], placenta [9], endometrium [10] and liver [11]. Particularly well described is the localization and respective function of pendrin in the thyroid gland, inner ear and kidney. In the thyroid gland, pendrin is expressed exclusively at the apical membrane of thyroid follicular cells [2]. Several points of evidence indicate that the pendrin transporter is crucially involved in follicular iodide transport, i.e. (i) its localization, (ii) the iodide organification defect presented by patients with Pendred syndrome [12] and (iii) a number of functional studies in heterologous expression systems (that will be reviewed here). In the inner ear, pendrin is expressed in the epithelium of the endolymphatic sac and duct [5, 13], on the apical membrane of transitional cells in the saccule, utricle, ampulla [5], and in a variety of diverse cell types in the cochlea (inner and outer hair cells, Deiter's cells, Claudius cells, spiral ligament, spiral ganglion, spiral prominence, external sulcus cells) [5, 14-17]. In these compartments, pendrin plays a crucial role in conditioning endolymph pH and ion composition [18]. In the kidney, pendrin is expressed on the apical membrane of β and non-α, non-β intercalated cells of the **d**istal **c**onvoluted **t**ubule (DCT), **c**ortical **c**ollecting **d**uct (CCD) and **co**n**n**ecting **t**ubule (CNT) [3, 19, 20] where it exerts a role in pH homeostasis [21] and blood pressure regulation [22, 23]. The physiological role of pendrin in other tissues is less well understood and deserves further investigation.

Transport properties of wild type pendrin

The thyroid

Pendrin was described as a member of the multifunctional transporters SLC26 [24] family, and after its discovery, it was assumed that pendrin transports sulphate. However, later studies in over-expression systems failed to demonstrate that pendrin can transport sulphate or other divalent anions [25, 26]. In addition, Kraiem et al. found that sulphate transport in thyrocytes obtained from Pendred syndrome patients was not defective [27]. Thereafter, it was shown that pendrin can transport iodide [25], and could therefore be involved in mediating apical iodide efflux from the thyroid cell into the follicular lumen [28, 29]. As time progressed, it became increasingly evident that pendrin acts as an electroneutral, i.e. non-rheogenic [30] iodide/chloride (I^-/Cl^-) exchanger with a 1:1 stoichiometry [31] and preference for iodide over other anions [32, 33].

It is noteworthy that some investigators assume that pendrin might also secrete bicarbonate into the thyroidal follicle, since thyroid follicular transepithelial potential and pH are reduced in pendrin knock-out mice [34]. For the supporting evidence as well as arguments questioning the role of pendrin in mediating iodide efflux in thyrocytes, see the reviews by Twyffels et al. [35] and Bizhanova et al. [36] in this Special Issue.

The kidney

Heterologous overexpression studies also demonstrated that pendrin can function as a chloride/hydroxide (Cl^-/OH^-) or chloride/bicarbonate (Cl^-/HCO_3^-) exchanger [37]. Studies using pendrin knock-out mice led to the conclusion that one role of pendrin in the kidney is bicarbonate secretion [3, 21]. Accordingly, pendrin expression was significantly increased following oral bicarbonate loading in mouse [38] and rat [39], and downregulated during metabolic acidosis in rat [39-41] and rabbit [42]. Patients with Pendred syndrome have normal renal function and do not display abnormalities in acid-base metabolism under basal conditions. This indicates that other chloride-base exchangers compensate for the loss of function of pendrin [43]. Recently however, two interesting case reports of severe metabolic alkalosis in patients with Pendred syndrome indicated that pendrin may play a role in protecting against metabolic alkalosis in the context of intercurrent illness [44] or pharmacological therapy [45].

Pendrin also plays a role in chloride reabsorption, since it is downregulated in response to high chloride intake and upregulated in response to chloride depletion induced by furosemide [46] or sodium chloride (NaCl) restriction [47, 48]. Accordingly, major changes in pendrin protein expression were found in experimental models that are associated with altered renal chloride transport [49].

Recently, it was proposed that pendrin in the kidney also plays a role in the maintaining of iodide balance, particularly under high water intake [50]; however, the mechanism by which pendrin drives renal iodide reabsorption in cases of increased water intake remains to be elucidated.

The inner ear

Similarly to the kidney, pendrin acts as a Cl^-/HCO_3^- exchanger in the inner ear, controlling the pH of the endolymphatic fluid. Earlier studies in pendrin knock-out mice, which are completely deaf and also display signs of vestibular dysfunction, revealed a severe endolymphatic dilatation after embryonic day 15, reminiscent of that seen radiologically in deaf individuals with pendrin mutations and EVA. Additionally, in the second postnatal week, severe degeneration of sensory cells in the Corti organ and vestibular maculae, and malformation of otoconia and otoconial membrane occurred. These observations provided important clues for understanding the cause of deafness and vestibular dysfunction in these mice and, possibly, in patients with Pendred syndrome [51]. Further studied revealed that pendrin is crucial in maintaining the endocochlear potential, albeit without being directly involved in potassium secretion [5]. Later, Wangemann et al. clarified that the loss of the endocochlear potential observed in the Pendred syndrome mouse model is due to a loss of expression of the potassium channel KCNJ10 [15]. The same group successively published a series of detailed studies on the role of pendrin in the different inner ear compartments using pendrin knock-out mice. These studies revealed a reduction in pH and utricular endolymphatic potential, and an increase in the endolymph calcium concentration, possibly due to the inhibition of pH-sensitive **t**ransient **r**eceptor **p**otential **v**anilloid (TRPV) 5 and TRPV6 cation channels [52]. These findings might explain the vestibular dysfunction observed in Pendred syndrome patients. A similar mechanism could induce the observed cochlear sensory hair cell degeneration, which, as a consequence, leads to deafness [18]. It was also hypothesized that a loss of bicarbonate secretion could hamper fluid reabsorption in the endolymphatic sac, an event eventually leading to cochlear enlargement and again, deafness [53]. All of the described functional derangements observed in the inner ear of pendrin knock-out mice would hence be associated with a decrease in endolymphatic bicarbonate secretion. Beside pendrin knock-out mice, also the use of genetically modified mice bearing pendrin mutations is a powerful tool in understanding the etiology of the pathological conditions related to pendrin malfunction. The *Slc26a4^{loop}* mouse, that was generated by N-**e**thyl N-**n**itroso**u**rea (ENU) mutagenesis bearing the homozygous loss of function mutation S408F, led to the discovery of new inner ear pathology that has complemented the work on the *Slc26a4* knock-out mouse with its novel phenotypic variation. Recently, we found dramatic changes in the composition, size, and shape of otoliths within the utricle and saccule of *Slc26a4^{loop}* mouse, possibly as a consequence of the deregulation of the endolymphatic pH [54].

The airways

Predominant pendrin expression in the airways was not discovered until 2005, when Kuperman et al. described upregulation of the transporter mRNA in the lungs of 3 separate murine asthma models [55]. Since the initial observation of pendrin expression in the bronchial epithelium, a multitude of studies have followed in which the transporter has been associated with increased antimicrobial activity in the **a**irway **s**urface **l**iquid (ASL) [6], mucus production [56] and regulation of the ASL thickness [57]. These recent data underscore a role for pendrin in respiratory distresses including allergy, rhinovirus infection, bronchial asthma and **c**hronic **o**bstructive **p**ulmonary **d**isease (COPD). For more detailed information regarding pendrin and the airways, see the Review by Nofziger et al. in this Special Issue [58].

The role of pendrin in human pathology

Diseases linked to pendrin malfunction

Mutation of the pendrin gene can lead to syndromic and non-syndromic hearing loss with **e**nlarged **v**estibular **a**queduct (EVA) [59]. Pendred syndrome, the most common form of syndromic deafness [60], was originally described more than one century ago as a combination of deafness and goiter unrelated to environmental factors [61]. Pendred syndrome is an autosomal recessive disease characterized by bilateral sensorineural deafness and a partial iodide organification defect disclosed by a positive perchlorate discharge test [62], even in the absence of overt goiter. Deafness in Pendred syndrome is usually severe to profound with an early onset, or fluctuating and progressive, and seldom occurring later in life or after head trauma [63]. Deafness is associated with a malformation of the inner ear, i.e. an EVA, accompanied by an enlarged endolymphatic sac and duct, or Mondini

cochlea, and can be diagnosed by computed tomography (CT) or magnetic resonance imaging (MRI) of the temporal bone [64]. By definition, ns-EVA is a condition where deafness due to inner ear malformations is not associated with thyroid dysfunction (the perchlorate discharge test in these patients is negative), and can be found in patients with zero, one [65, 66] or two mutations in the pendrin gene [67-69]. Whether or not ns-EVA can be associated with two mutations of the pendrin gene is a matter of debate. Pryor et al. found a strong correlation between Pendred syndrome and two mutant *SLC26A4* alleles, while ns-EVA correlated with zero or one mutant *SLC26A4* alleles. [65]. This study therefore suggests that biallelic *SLC26A4* mutations are consistently associated with a positive perchlorate test, and hence, with Pendred syndrome. As mentioned earlier, other studies indeed suggested that ns-EVA can be associated with two mutations of the pendrin gene [67-69]. Nevertheless, it is noteworthy that in the study of Azaiez et al. there was no definitive assessment of the thyroid phenotype, that was defined as "palpable goiter or abnormal perchlorate discharge test" [67]. Albert et al. reported several cases of biallelic *SLC26A4* mutations with a normal perchlorate discharge test [68]. Unfortunately, these Authors did not specify the exact cut-off value for considering the test as positive. Tsukamoto et al. reported several cases of ns-EVA with biallelic *SLC26A4* mutations; once again, the criterion for the assessment of the thyroid phenotype was not precisely described. These Authors defined patients with Pendred syndrome as "those having either a palpable goiter or abnormal perchlorate discharge test" [69]. In conclusion, these studies seem to indicate that although biallelic *SLC26A4* mutations are often associated with Pendred syndrome, cases of ns-EVA associated with biallelic *SLC26A4* mutations can also occasionally be found.

Thyroid dysfunction in Pendred syndrome is variable, as is the presence and dimension of goiter [70]. Patients are usually euthyroid, or may present modestly elevated serum levels of **t**hyroid **s**timulating **h**ormone (TSH); often **t**hyro**g**lobulin (TG) is significantly increased [71] and occasionally hypothyroidism may develop [72]. Pendred syndrome is typically linked to biallelic mutations (as homozygosity in inbred families, or as compound heterozygosity, Fig. 1) occurring in the pendrin coding region. In addition, cases of Pendred syndrome/non-syndromic EVA have been reported with mutations occurring in the consensus binding region for the transcription factor **fo**rkhead bo**x** (FOX) I1 (present in the promoter region of pendrin) [73], and double heterozygous mutations in the *FOXI1* coding region or in the potassium channel KCNJ10, which participates in the generation of the endocochlear potential [74] (Fig. 1).

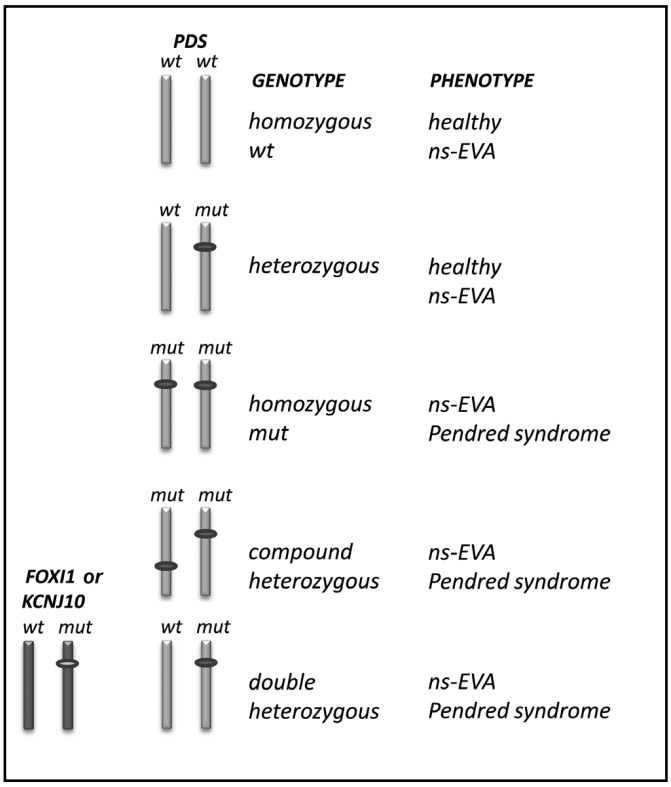

Fig. 1. Nomenclature used in this review; ns-EVA could be found in individuals with zero, one, or two (as homozygous or compound heterozygous) mutations of the pendrin gene, or in individuals bearing mutations in the pendrin gene and another functionally related gene (double heterozygous). Pendred syndrome could only be found in individuals with two (as homozygous or compound heterozygous) mutations of the pendrin gene or in individuals bearing mutations in the pendrin gene and another functionally related gene (double heterozygous).

Pendrin hyper-function and blockers

As previously mentioned, chloride reabsorption via pendrin at the level of the kidney could contribute to the pathogenesis of hypertension. Indeed, expression and activity of the transporter are upregulated by aldosterone analogues [75] and angiotensin II [76]. Accordingly, pendrin knock-out mice are protected against aldosterone analogue -induced hypertension [75]. Pendrin may thus represent a potential target for blood pressure control [22]. In the airways, pendrin has been associated with mucus production [56] and is upregulated upon stimulation with pro-inflammatory cytokines [6, 56, 57, 77, 78]. The chloride reabsorption via pendrin could reduce the airway surface liquid thickness [57], exacerbating, together with

mucus overproduction, the symptoms of asthma and COPD [77]. In such pathological conditions, blocking or reducing pendrin activity with the use of selective drugs could be beneficial. Moreover, very recently pendrin allelic variants with a modest, but significant, gain of function have been identified [79]. These hyperfunctional mutants could be genetic modifiers contributing to the severity of the phenotype of hypertension, asthma and COPD. A considerable effort has been devoted in characterizing the pharmacological profile of this transporter; nevertheless, no selective, potent, non-toxic inhibitors have been identified so far. Pendrin shows an unusual inhibitor profile when compared to other anion exchangers. In some heterologous overexpression systems, pendrin seems to be scarcely sensitive [25] or even resistant [32] to the addition of DIDS (4,4'-Diisothiocyano-2,2'-stilbenedisulfonic acid), a well-known blocker of Cl^-/HCO_3^- exchangers [80]. Similarly, other inhibitors, such as furosemide and probenecid, only showed a partial effect, even at high concentrations [25]. In contrast, pendrin mediated chloride transport is sensitive to the chloride channel blocker NPPB (5-Nitro-2-(3-phenylpropylamino)benzoic acid) [32]. Interestingly, the most potent pendrin inhibitor at present seems to be the nonsteroidal anti-inflammatory drug niflumic acid, which, at the concentration of 10^{-4} M, was able to reduce pendrin associated chloride uptake by ~70% [32]. Accordingly, Pedemonte et al. screened a multiple compounds library and found that only niflumic acid blocked pendrin associated anion transport [6]. The identification of potent and selective inhibitors of pendrin deserves further investigation.

Molecular and functional characterization of pendrin allelic variants

Functional tests

More than 170 mutations within the pendrin gene have been identified so far (http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm). The majority (~64%) of these mutations are single nucleotide changes leading to amino acid substitutions, followed by ~16% leading to amino acid insertions or deletions, ~13% affecting splicing sites, and ~6% leading to premature truncations of the protein. Functional tests of pendrin mutations identified as allelic variants in patients with Pendred syndrome or ns-EVA revealed that the pathological phenotype is the consequence of a reduction or loss of function of pendrin-driven ion transport [81]. In the first study aimed at determining the transport activity of pendrin mutants, Scott et. al. measured the uptake of radiolabeled iodide and chloride in *Xenopus laevis* oocytes injected with wild-type (WT) or mutated pendrin cRNA [81]. Taylor et al. successively measured the activity of mutated pendrin using radiolabeled iodide efflux assays in human cells transfected with WT and mutated pendrin [82]. The use of human cells instead of a non-mammalian system for chloride uptake [32] and iodide efflux [83-85] studies was a considerable improvement, since they provided a more physiologically relevant environment to determine pendrin activity. A further improvement was the use of polarized mammalian cells by Gillam et al. These authors developed a functional test with polarized **M**adin-**D**arby **c**anine **k**idney (MDCK) cells loaded with radiolabeled iodide by means of the $\mathbf{Na^+/I^-}$ **s**ymporter (NIS) in a double-chamber system, allowing the measurement of iodide efflux *via* WT pendrin [29]. Determination of pendrin transport activity using radioisotopes is advantageous since radiolabeled iodide can be used at relatively low concentrations (close to the micromolar concentration present in the cytoplasm of the thyrocyte); however, these studies are simultaneously burdened by the use of radioactivity. In 2006, we described a non-radioactive, fluorescence-based assay suitable for the measurement of pendrin function [31, 54, 70, 86]. A further improvement of this technique was acquired with the use of an **e**nhanced **y**ellow **f**luorescent **p**rotein (EYFP) isoform (EYFP H148Q/I152L) that is more sensitive to the intracellular iodide concentration when compared to chloride, although not specific (pKa for iodide and chloride 3 and 88 mM respectively, at pH 7.5) [87]. In addition, the Cl^-/HCO_3^- and Cl^-/OH^- exchange activity of WT pendrin and its mutants can be measured by means of the pH_i-sentitive dye BCECF [88]. It is worth to note that very few studies compared the I^-/Cl^- and the Cl^-/HCO_3^- exchange activity of the same mutants [79, 89-91]. Besides in heterologous overexpression systems, the activity (iodide efflux) of the mutated pendrin has been characterized in primary thyrocyte cultures from a patient with Pendred syndrome [92]. This model mimics the *in vivo* situation most adequately, however, this approach can only be seldom applied. Table 1 shows all pendrin mutations identified so far in patients with Pendred syndrome or ns-EVA for which a functional test or the determination of the subcellular localization has been reported. The topology of the mutations for which a functional characterization has been performed is also indicated on the putative model of pendrin (Fig. 2) that

Mutation	Activity compared to WT	Nucleotidic substitution	Pathology	Localization	Function	Rescue	SNPs database[20]	Pathology[21]	Reference #
S28R	-	84C>A	PS	PM[6]	loss of chloride uptake[11] loss of chloride and iodide transport[12]				[93] [31]
		82A>C	EVA	intracellular[7]	loss of Cl^-/HCO_3^- exchange activity[13]	Yes[17]			[88]
E29Q	-	85G>C	EVA		reduction of the chloride and iodide transport[12]		Yes	EVA	[86]
P70L	-	209C>T			loss of chloride and iodide transport[12]		Yes		[79]
V88I	+	262G>A	EVA (+R409H)		increased chloride and iodide transport[12] Cl^-/OH^- exchange activity not reduced[13] increased iodide efflux[15]				[86] [79]
FS93>96X[1]+ C416-1G_A[2]	-	279delT	PS	intracellular[6,8]	intracellular iodide retention (primary thyrocytes culture)[14,15]				[92]
G102R	-	304G>A	PS	ER[9]	loss of iodide efflux[15]				[82]
L117F	=	349C>T	EVA	PM[9]	normal iodide efflux[15]		Yes		[82]
P123S	-	367C>T	PS	intracellular[8]	loss of Cl^-/I^- exchange activity[15]	Yes[18]			[85]
V138F	-	412G>T	PS	ER[9]	loss of iodide efflux[15]		Yes	PS	[82]
P140H	-	419C>A	EVA		loss of chloride and iodide transport[12]				[86]
P142R	-	425C>G	EVA	intracellular[7]	loss of Cl^-/HCO_3^- exchange activity[13]	+/-[17]			[88]
M147V	-	439A>G	EVA EVA	intracellular[7] intracellular[8]	loss of Cl^-/HCO_3^- exchange activity[13] loss of Cl^-/I^- exchange activity[15]		Yes[17] Yes[18]		[88] [85]
M147T		441T>C		intracellular[6,8]					[104]
S166N	=	497G>A	EVA	PM[7]	normal Cl^-/HCO_3^- exchange activity[13]				[88]
G209V	-	626G>T	PS, EVA	PM[9]	severe reduction of Iodide efflux[15]		Yes	EVA	[82]
L236P	-	707T>C	PS PS PS, EVA	ER[9] ER[9] intracellular[7]	loss of iodide efflux[15] loss of chloride and iodide uptake[11,14] loss of Cl^-/HCO_3^- exchange activity[13] loss of Cl^-/I^- and Cl^-/HCO_3^- exchange activity[16]	 No[17, 19]	Yes	PS	[82] [81] [96] [88] [89]
V239D	-	716T>A	EVA	ER[10]	severe reduction of chloride and iodide transport[12] loss of Cl^-/OH^- exchange activity[13]		Yes	pathogenic	[95] [91]
V250A	=	749T>C	EVA		normal Cl^-/HCO_3^- and Cl^-/I^- exchange activity[16]				[90]
D266N	=	796G>A	EVA		normal Cl^-/HCO_3^- and Cl^-/I^- exchange activity[16]		Yes		[90]
FS297>302X	-	890delC	PS		loss of iodide efflux[15]				[84]
P301L	-	902C>T			loss of chloride and iodide transport[12]		Yes		[79]
E303Q	-	907G>C	EVA	PM[8]	loss of Cl^-/I^- and Cl^-/HCO_3^- exchange activity[16]				[90]
FS306>309X	-	916insG	PS	intracellular[9]	loss of iodide efflux[15]				[29]
G334V 335X	-	1001G>T	EVA		loss of chloride and iodide transport[12] loss of Cl^-/OH^- exchange activity[13]				[91]
F335L	-	1003T>C	EVA	PM[9]	reduction of Cl^-/I^- and Cl^-/HCO_3^- exchange activity[16]		Yes	EVA	[89]
F354S	=?	1061T>C	EVA		mild reduction of Cl^-/HCO_3^- exchange activity[16] chloride and iodide transport not reduced[12] Cl^-/OH^- exchange activity not reduced[13] iodide efflux not reduced[15]		Yes		[90] [79]

Mutation	Activity compared to WT	Nucleotidic substitution	Pathology	Localization	Function	Rescue	SNPs database[20]	Pathology[21]	Reference #
K369E	=	1105A>G	EVA	PM[8]	normal Cl^-/I^- exchange[15]		Yes	EVA	[85]
A372V	-	1115C>T	PS, EVA	intracellular[8]	loss of Cl^-/I^- exchange activity[15]	No[18]	Yes	EVA	[85]
E384G	-	1151A>G	PS EVA	ER[9] intracellular[7]	loss of chloride and iodide uptake[11,14] loss of Cl^-/HCO_3^- exchange activity[13] loss of Cl^-/I^- and Cl^-/HCO_3^- exchange activity[16]	 No[17]	Yes	PS	[81] [96] [88] [89]
N392Y	-	1174A>T	PS	intracellular[8]	loss of Cl^-/I^- exchange activity[15]	No[18]			[85]
V402M	-	1204G>A	EVA	intracellular[9]	loss of Cl^-/HCO_3^- and Cl^-/I^- exchange activity[16]				[89]
R409H	-	1226G>A	PS	partially PM[6]	loss of iodide efflux[15] reduction of iodide and chloride transport[12]		Yes	probably pathogenic	[84] (unpublished data)
R409H/V88I[3]	-	1226G>A 262G>A	EVA		reduction of the chloride and iodide transport[12]				[86]
T410M	-	1229C>T	EVA	ER[9]	loss of iodide efflux[15]		Yes	Pathogenic	[82]
Q413P	-	1238A>C	PS		loss of chloride and iodide transport[12]				[86]
T416P	-	1246A>C	PS EVA	ER[9] intracellular[7]	loss of chloride and iodide uptake[11,14] loss of Cl^-/HCO_3^- exchange activity[13] loss of Cl^-/HCO_3^- and Cl^-/I^- exchange activity[16]	 +/-[17]	Yes	PS	[81] [96] [88] [89]
G424D	-	1271G>A	PS		reduction of the chloride and iodide transport[12]				[86]
L445W		1334T>G	EVA/PS	intracellular[9] intracellular[6,8]			Yes	PS	[89] [104]
Q446R	-	1337A>G	EVA	ER[9]	loss of iodide efflux[15]				[82]
V480D	-	1440T>A	EVA		reduction of chloride and iodide uptake[11,14]				[81]
T485R	-	1454C>G	PS		reduction of the chloride and iodide transport[12]				[86]
I487YFSX39 (526X)	-	1458_1459insT	EVA	ER[10]	loss of chloride and iodide transport[12] loss of Cl^-/OH^- exchange activity[13]				[94] [91]
I490L	-	1468A>C	EVA		mild reduction of chloride and iodide uptake[11,14]				[81]
G497S	-	1489G>A	EVA EVA	intracellular[7]	strong reduction of chloride and iodide uptake[11,14] loss of Cl^-/HCO_3^- exchange activity[13]	 +/-[17]	Yes	pathogenic	[81] [88]
I490L G497S[5]	-	1468A>C 1489G>A	EVA		strong reduction of chloride and iodide uptake[11,14]				[81]
Q514K	-	1541C>A	EVA, PS		loss of chloride and iodide transport[12]		Yes	EVA	[86]
FS523>548X	-	1561_1571CTTGGAA TGGC	PS		loss of chloride and iodide transport[12]				[70]
Y530H		1588T>C	PS	intracellular[9]			Yes	PS	[89]
Y530S		1589A>C	EVA	intracellular[9]					[89]
Y556C	-	1667A>G	PS	partially PM[9]	loss of iodide efflux[15]				[82]
C565Y	=?	1694G>A	EVA	PM[9] PM[8]	reduction of Cl^-/I^- and Cl^-/HCO_3^- exchange activity[16] normal Cl^-/I^- exchange activity[15]		Yes	probably pathogenic	[89] [85]
L597S	=?	1790T>C	controls only EVA	PM[9]	normal chloride and iodide transport[12] reduction of Cl^-/I^- and Cl^-/HCO_3^- exchange activity[16]		Yes	probably non pathogenic	[86] [89]
V609G	-	1826T>C	NSHL		reduction of chloride and iodide transport[12] reduction of iodide efflux[15]		Yes	non pathogenic	[79]

Mutation	Activity compared to WT	Nucleotidic substitution	Pathology	Localization	Function	Rescue	SNPs database[20]	Pathology[21]	Reference #
E625X	-	1873G>T	EVA	intracellular[7]	loss of Cl^-/HCO_3^- exchange activity[13]	No[17]			[88]
V653A	-	1958T>C	EVA		reduction of chloride and iodide uptake[11,14]				[81]
S657N	-	1970G>A	EVA	intracellular[8]	loss of Cl^-/I^- exchange activity[15]	Yes[18]			[85]
S666F	-	1997C>T	EVA	intracellular[8]	loss of Cl^-/I^- exchange activity[15]	No[18]			[85]
F667C	-	2000T>C			loss of chloride and iodide transport[12]		Yes	PS	[79]
G672E	-	2015G>A	PS	partially PM[9]	loss of iodide efflux[15]		Yes	pathogenic	[82]
L676Q	-	2027T>A	PS EVA	intracellular[9] intracellular[7]	loss of iodide efflux[15] loss of Cl^-/HCO_3^- exchange activity[13]	+/-[17]	Yes	pathogenic	[29] [88]
D687Y	-	2059G>T			reduction of chloride and iodide transport[12]		Yes		[79]
D697A	-	2090A>C	EVA	intracellular[8]	reduction of Cl^-/I^- exchange activity[16]		Yes		[90]
K715N	-	2145G>T	EVA	intracellular[8]	reduction of Cl^-/I^- exchange activity[16]				[90]
T721M	-	2162C>T	EVA	intracellular[8]	loss of Cl^-/I^- exchange activity[15]	No[18]	Yes	PS, EVA	[85]
H723R	-	2168A>G	PS	intracellular[7] intracellular[8]	loss of Cl^-/HCO_3^- exchange activity[13] loss of Cl^-/I^- exchange activity[15]	Yes[17,19] Yes[18]	Yes	PS	[88] [85]
D724G	-	2171A>G	EVA		loss of chloride and iodide transport[12]				[86]
E737D	-	2211G>C	EVA	intracellular[8]	reduction of Cl^-/I^- and Cl^-/HCO_3^- exchange activity[16]				[90]
G740S	+	2218G>A	NSHL		increased chloride and iodide transport[12] increased Cl^-/OH^- exchange activity[13] increased iodide efflux[15]		Yes		[79]
M775T	-	2324T>C	EVA	PM[9]	reduction of Cl^-/I^- and Cl^-/HCO_3^- exchange activity[16]				[89]
R776C	=?	2326C>T	TPO mut. EVA	PM[9]	normal iodide efflux[15] reduction of Cl^-/I^- and Cl^-/HCO_3^- exchange activity[16]		Yes	probably non pathogenic	[83] [89]

Table 1. Summary of all the pendrin allelic variants for which the functionality or the subcellular localization is known as of now. For some mutants, the possibility to rescue the function with chemical or physical chaperones is reported. If the allelic variant is reported in the single nucleotide polymorphisms (SNPs) database is also indicated. +, gain of function; -, reduction or loss of function; =, benign polymorphisms; =?, controversial; the corresponding allelic variant may be hypofunctional; [1]originally reported as: Ser93ArgfsX3; [2]acceptor splice site mutation; [3]both mutations are present on the same chromosome; [4]originally reported as: Ile487TyrfsX39; [5]mutations are present on different chromosomes; the respective mutated proteins were co-expressed; [6]western blot; [7]N-glycosylation; [8]confocal microscopy, immunofluorescence; [9]GFP-fusion protein; [10]YFP-fusion protein; [11] $^{36}Cl^-$ uptake; [12]fluorometric method; [13]measure of the pH_i (BCECF); [14] $^{125}I^-$ uptake; [15] $^{125}I^-$ efflux; [16] $^{36}Cl^-$ efflux (rate constant evaluation); [17]low temperature; [18]10 mM salicylate; [19]Na-butyrate; [20]http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?showRare=on&chooseRs=all&locusId=5172&mrna=NM_000441.1&ctg=NT_007933.15&prot=NP_000432.1&orien=forward&refresh=refresh, accessed on the 14th of September 2011; [21]http://omim.org/entry/605646; del: deletion; ins: insertion; PS: Pendred syndrome; EVA: enlarged vestibular aqueduct; NSHL: non-syndromic hearing loss; TPO: thyroperoxidase; PM: plasma membrane; ER: endoplasmic reticulum; GFP: green fluorescent protein; YFP: yellow fluorescent protein; BCECF: 2',7'-bis-(2- carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester.

we recently suggested [93]. It is noteworthy that this model is very different from other predictions and that there are no experimental data proving any of the suggested models aside from findings suggesting that both the amino and the carboxyl termini are intracellular [93]. The functional tests allowed the identification of pendrin allelic variants with a reduction of loss of function respect to WT; for these allelic variants a pathogenic potential can likely be assumed, and they are thereafter referred as "mutations". In addition, allelic variants whose function is not significantly different from WT were found, and are thereafter referred as "benign polymorphisms". Moreover, very recently, allelic variants showing a moderate gain of function were identified [79].

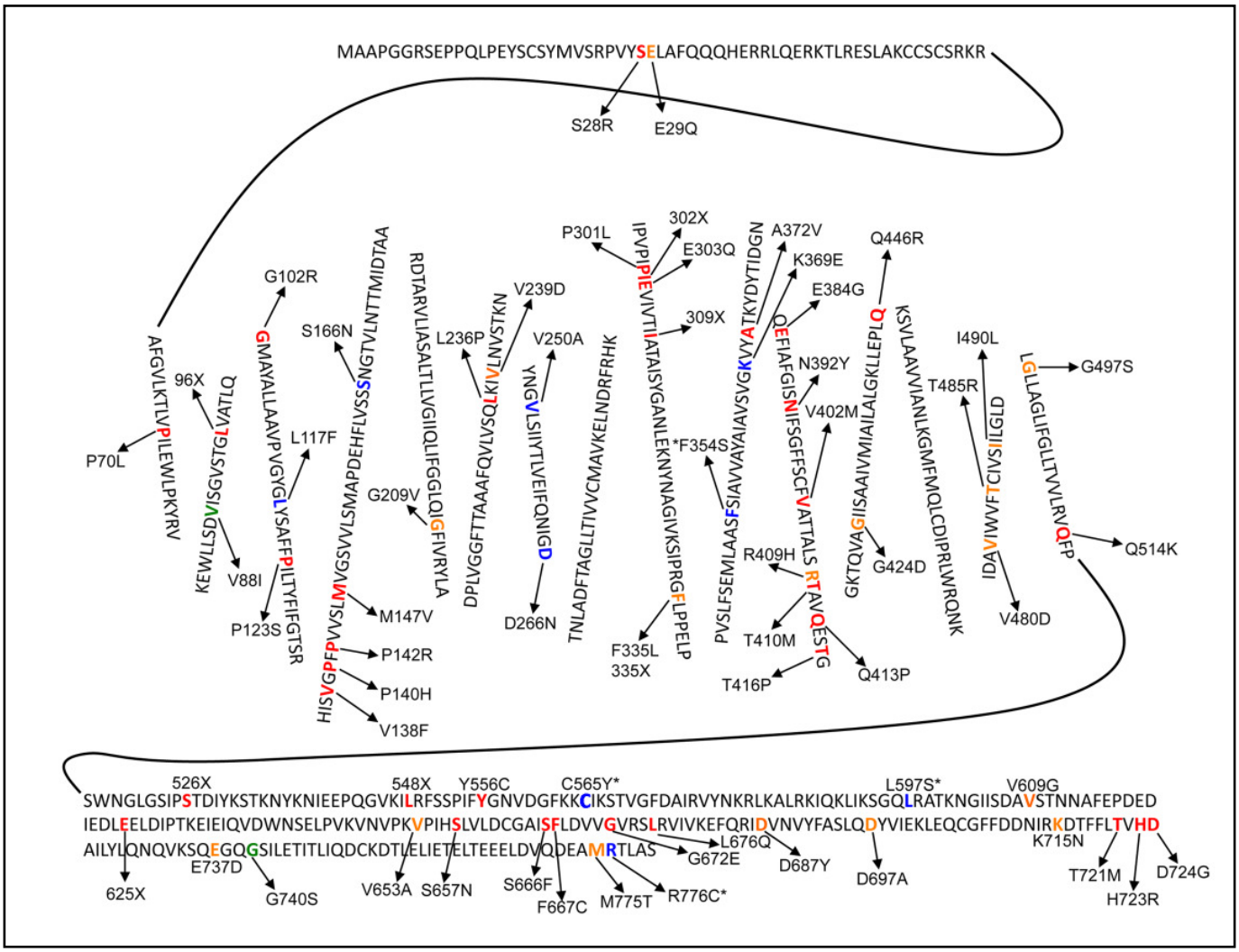

Fig. 2. Topology of pendrin allelic variants that have been functionally characterized as of now; the putative model of pendrin structure is taken from [87]. Amino acids that are affected by loss of function mutations are indicated in red, reduction of function are indicated in orange. Light blue indicates amino acid substitutions without functional impact, i.e. those mutations for which the activity is indistinguishable from WT (benign polymorphisms). Green indicates allelic variants with a modest gain of function. The extension 'X' indicates the mutations leading to premature truncation of the protein. Asterisks denote those allelic variants that are indicated as benign polymorphisms in this context, but with ambiguous function (they may be hypofunctional).

Intracellular localization

Localization studies revealed that mutated pendrin proteins are often retained in intracellular compartments and are unable to reach the plasma membrane [94-96]; as a consequence, the transport function is impaired [82, 85, 88-90, 92, 96]. These mutations most likely cause misfolding of the protein, with defective trafficking and subsequent degradation ("processing" defect). Some mutated proteins, however, reach the plasma membrane and show impaired transport function [32, 82, 89, 90]. These proteins are of particular interest, because the mutations possibly change ion binding or regulatory domains (Fig. 3). Several amino acid substitutions (F335L, C565Y, L597S, M775T, R776C), most of which are located in the C terminus of the transporter, do not affect the targeting of the protein to the plasma membrane; their function is only slightly impaired compared to WT. They could, therefore, be classified as hypofunctional allelic variants [89]. Moreover, the function of C565Y, L597S, and R776C is controversial, as these allelic variants were classified as benign polymorphisms by other Authors [83, 85, 86] (Fig. 3). We conclude that these mutations probably do not affect important regions for ion binding or important regulatory domains of the transporter. The G209V [82] and E303Q [90] mutations also reach the plasma membrane, however, their function is severely impaired, indicating that these mutations do not impair trafficking, but might alter binding of the transported ions [90].

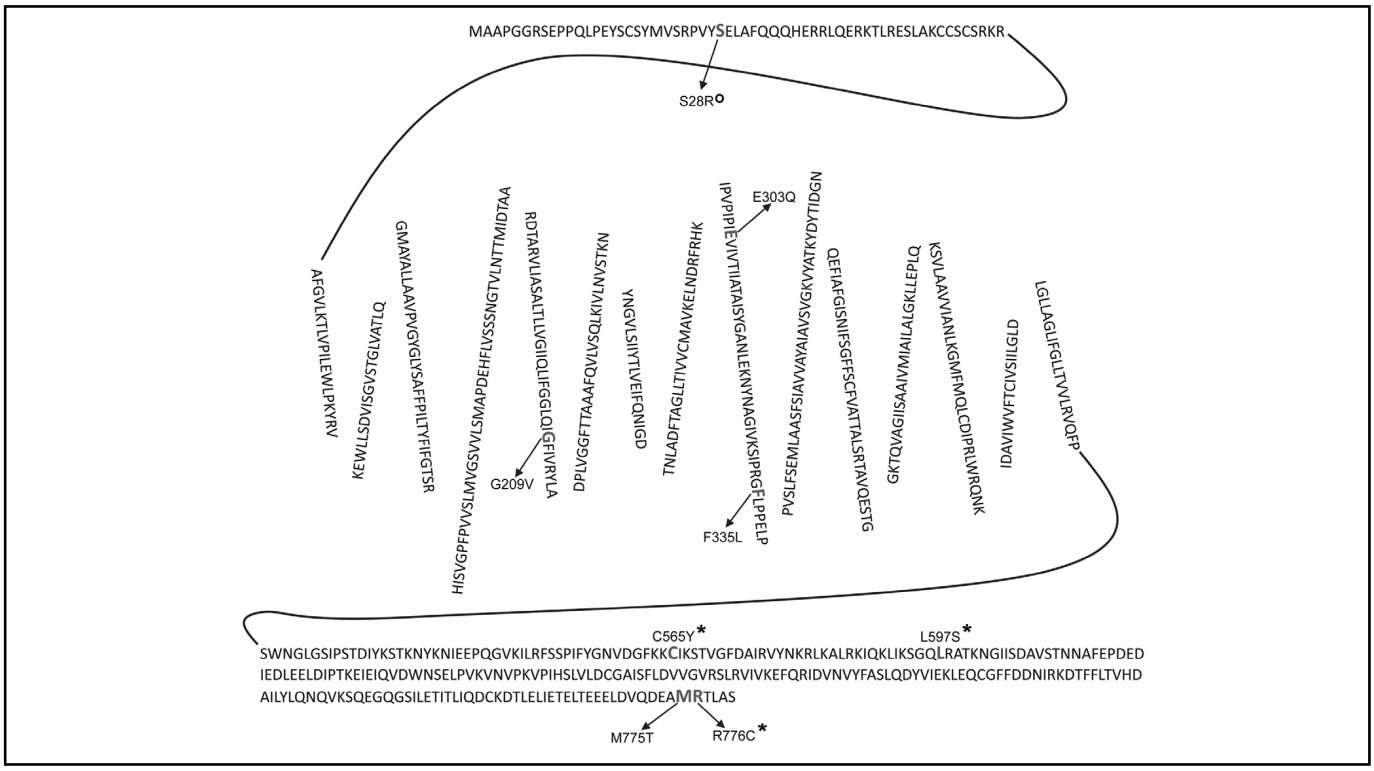

Fig. 3. Topology of the pendrin mutations with loss or reduction of function and normal trafficking to the plasma membrane; the putative model of pendrin structure is taken from [87]. Asterisks indicate those allelic variants whose functional alteration remains controversial. ° indicates a mutation for which the targeting to the plasma membrane is ambiguous.

Restoring transport function

Since the majority of pendrin mutants show a "processing" defect (see above), it is plausible that targeting of the mutants to the plasma membrane could be assisted by physical or pharmacological chaperones. Yoon et al. demonstrated that the plasma membrane targeting of some such mutants (S28R, M147V, H723R) (Table 1) could be rescued by growing the transfected cells at low temperature; interestingly, the Cl^-/HCO_3^- exchange activity of the mutant H723R, which is the most common disease-associated mutation in East Asians [97], was also restored after low-temperature incubation or Na^+-butyrate treatment [88]. Furthermore, Ishihara et al. demonstrated that 10 mM salicylate rescued the impaired trafficking of pendrin mutants P123S, M147V, S657N and H723R (Table 1) from the subcellular compartments to the plasma membrane, and restored transport activity (iodide efflux) [85]. Unfortunately, the response of the different pendrin mutants to chaperons is not identical; while some mutants are responsive to physical and chemical chaperones (such as M147V, H723R), others, such as L236P (the most common disease-associated mutation in Caucasians), A372V, E384G, N392Y, E625X, S666F and T712M, are not (Table 1) .

Benign polymorphisms

The functional characterization of pendrin mutants led to the identification of allelic variants for which the plasma membrane trafficking and transport function were not significantly impaired compared to WT (Table 1 and Fig. 2). At present, functional assays have shown that the transport activity of the following allelic variants is not significantly different compared to WT pendrin: L117F [82], S166N [88], V250A, D266N [90], F354S [79], K369E, C565Y [85], L597S [86] and R776C [83]. It is reasonable to assume that these mutations are benign polymorphisms, and that if they are found in patients with deafness and goiter, other genetic or environmental explanations should be considered in the diagnosis. However, others suggest that F354S [90], C565Y, L597S, R776C, despite retaining significant transport activity, should be considered hypo-functional and, if present in *trans* configuration (i.e. on a different allele of the pendrin gene) with a mutation, could display pathogenicity [89].

Gain of function mutations

V88I and G740S are allelic variants whose function is increased compared to WT [79, 86]. Given the aforementioned possible role of pendrin in the pathogenesis

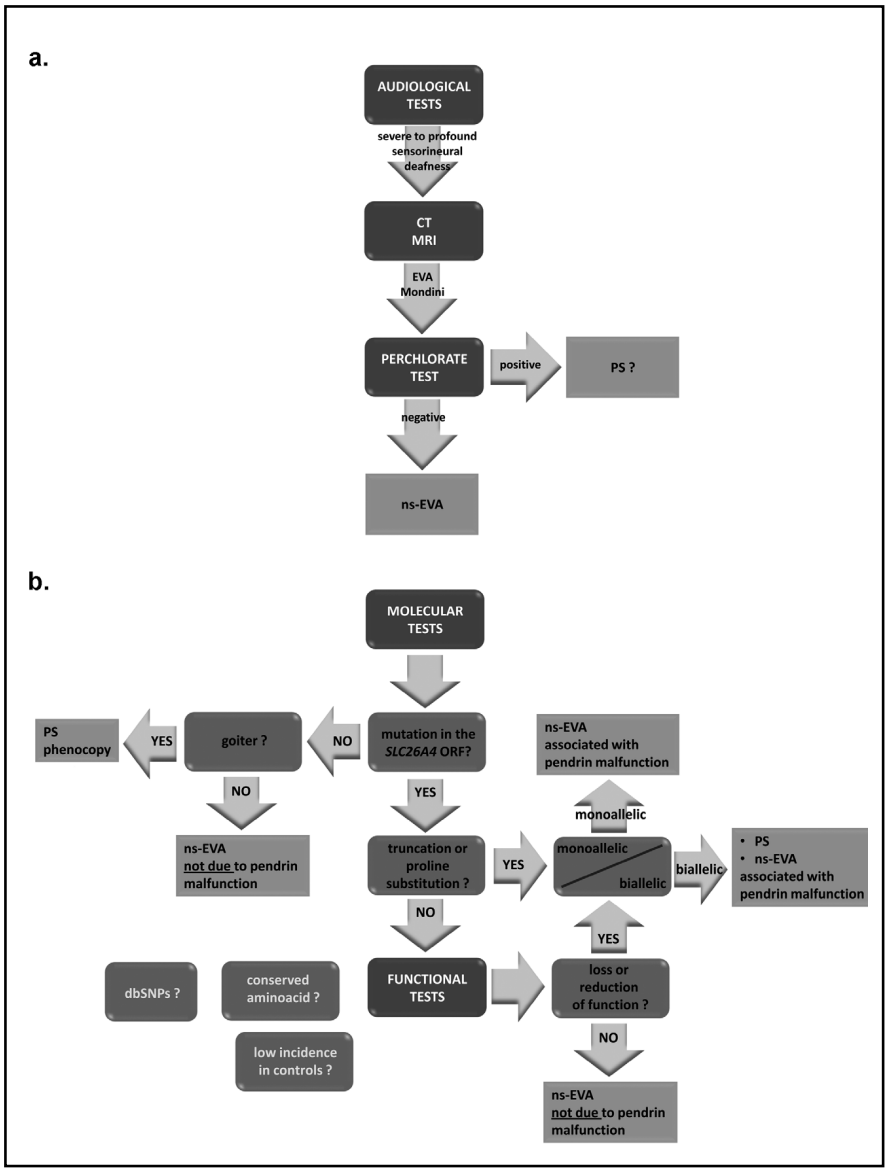

Fig. 4. Suggested algorithm for the diagnosis of Pendred syndrome/ns-EVA related to pendrin dysfunction. (a) if in a patient with severe to profound sensorineural deafness the **c**omputed **t**omography (CT) or **m**agnetic **r**esonance **i**maging (MRI) of the temporal bone disclose malformations of the inner ear such as an EVA or Mondini cochlea, with or without a positive perchlorate discharge test, the molecular screening (sequencing) of the pendrin gene (b) is recommended. The detection of a mutation in the *SLC26A4* ORF leading to a truncated protein or an amino acid substitution involving a proline is a strong indication that the allelic variant is a pathogenic mutation. In all the other cases, only a functional test could discriminate between pathogenic mutations and allelic variants that cannot be considered as genetic determinants for Pendred syndrome/ns-EVA. Monoallelic mutations with reduction or loss of function are associated with ns-EVA. Biallelic (homozygous, compound heterozygous or double heterozygous, see Fig. 1) mutations with reduction or loss of function are associated with Pendred syndrome or, occasionally, with ns-EVA. The presence of a specific allelic variant in the dbSNPs should not imply a benign polymorphism; similarly, the low incidence in the control population or the involvement of a highly conserved amino acid should not be considered as indications of impaired function, and hence, pathogenicity. PS: Pendred syndrome; ns-EVA: non-syndromic enlarged vestibular aqueduct; ORF: open reading frame.

of hypertension, COPD and asthma, these variants may contribute to the severity of the phenotype and/or exacerbations of compromised airway function. The mechanism conferring a gain of function to the transporter is not known: as the putative anion binding site was postulated to be in a different region of the molecule [90], these amino acid substitutions likely do not increase the affinity of the transporter for its substrates. As WT pendrin is a slow-folding protein [98], with substantial retention in the intracellular compartments [32], there is the possibility that amino acid substitutions V88I and G740S aid in folding of the protein, consequently increasing its targeting to the plasma membrane. Alternatively, these amino acid substitutions could improve the affinity of the transporter for not yet identified regulatory partners that may increase pendrin activity.

Genotype-phenotype correlation

Considerable effort has been devoted to correlate the type of mutation with the phenotype found in the respective patient (age of onset and degree of deafness, presence of goiter, etc.). It was previously proposed that loss of function mutations could confer Pendred syndrome, while mutations with residual transport could be associated with ns-EVA [81]. However, the identification of mutations common to both pathological conditions led to the exclusion of this hypothesis [69]. The correlation between the specific pendrin mutation and the clinical phenotype of the patient is difficult for the following reasons: (i) for a significant number of patients described in the literature, the perchlorate discharge test has not been performed, so that no discrimination between Pendred syndrome and ns-EVA

in the absence of overt goiter was possible, (ii) if the mutation is found in compound heterozygosity, only rarely have both detected alleles been characterized functionally, and (iii) seldom have both the I^-/Cl^- and Cl^-/HCO_3^- exchange activities been determined. It is important to note that I^-/Cl^- exchange, which is more relevant at the level of the thyroid gland, may be less affected by mutations when compared to Cl^-/HCO_3^- exchange, that is more relevant at the level of the inner ear [89, 90]. This fact could explain, at least in part, the lower penetrance of thyroid abnormalities than of EVA and hearing loss [90]. In addition, nutritional iodide intake [99] and epigenetic factors, including individual variations in WT or mutant pendrin expression levels, or proteins that could partially substitute for pendrin function should be considered.

The functional characterization of pendrin allelic variants may be a valuable help in the diagnosis of Pendred syndrome and ns-EVA due to pendrin malfunction

The diagnosis of Pendred syndrome and ns-EVA due to pendrin malfunction is challenging and may rely on the following tools (Fig. 4a): (i) the audiological examination, that should reveal severe to profound sensorineural deafness; (ii) the imaging of the temporal bone, that should reveal malformations of the inner ear, such as an EVA or Mondini cochlea, and an enlarged endolympatic sac when evaluated appropriately with MRI [64]; (iii) the perchlorate discharge test, that discloses a possible iodide organification defect and, in the absence of overt goiter, is essential for discriminating between Pendred syndrome and ns-EVA [65]. The existence of phenocopies of Pendred syndrome (i.e. patients displaying goiter and deafness unrelated to pendrin malfunction) [100-102], and the fact that ns-EVA could also be unrelated to pendrin mutations [65, 66], led to the conclusion that molecular tests (i.e. the sequencing of pendrin gene, Fig. 4b) are essential for the diagnosis of Pendred syndrome/ns-EVA due to pendrin malfunction [103]. However, the identification of one or two mutations in the pendrin gene is not sufficient to conclude that the detected mutation(s) is (are) the genetic cause of the phenotype of the patient. Indeed, the presence of functional, benign polymorphisms in some populations (Table 1 and Fig. 2) and the misclassification of these benign polymorphic variants as pathogenic alleles [89] can lead to erroneous classification. As a consequence, is essential that, whenever a mutation in the pendrin gene is found, its pathogenic potential is established.

Recently, we defined that the two parameters used so far to assess the pathogenic potential of a mutation, low incidence in the control population, and substitution of evolutionary conserved amino acids, are not always sufficient for defining the pathogenicity of pendrin allelic variants [86]. Indeed, a pathogenic mutation is expected to occur with lower frequency in the cohort of normal-hearing individuals with respect to the hearing-impaired cohort of patients. In contrast, in the Spanish population, we unexpectedly identified mutations with impaired function, hence most likely pathogenic, (E29Q, V609G, D724G) occurring with the same frequency in the cohort of hearing impaired patients and in the control group of normal hearing individuals [79, 86]. Similarly, the amino acid change F667C was identified in the control population only and not in deaf patients; despite that, the functional tests revealed that this allelic variant is a mutation with reduced function, and not a benign polymorphism, as expected from its incidence. In the same context, a pathogenic mutation is expected to affect conserved amino acids. However, we and other groups identified functional, benign polymorphisms affecting residues highly conserved among pendrin orthologs [93], such as F354S [79], K369E [85], and L597S [86]. Identifying new criteria for establishing the pathogenicity of an allelic variant is therefore crucial. As a general rule in this complex scenario, we previously observed a complete loss of function for all truncation mutations and mutations involving the substitution of a proline or a charged (acid or basic) amino acid [86]. As the effort of the functional characterization of pendrin allelic variants progressed, it became obvious that the involvement of a charged amino acid is not always sufficient to induce a detrimental effect on the ion transport. Indeed, the functionality of pendrin D266N and K369E is not reduced with respect to WT (Table 1 and Fig. 2). However, (i) truncation mutations and (ii) mutations involving the substitution of a proline always showed a complete loss of function (Table 1, Fig. 2 and 4b). In all the other cases, only a functional test allows for the discrimination between a pathogenic mutation and a benign polymorphism (Fig. 4b).

Conclusions

The diagnosis and the discrimination between Pendred syndrome and ns-EVA can be difficult because of the existence of Pendred syndrome phenocopies; in this view, clinical and radiological studies could be corroborated by genetic and molecular studies. In

performing genetic studies, the possibility that the mutation could affect the pendrin promoter, intronic regions or coding regions of functionally related genes (*FOXI1, KCNJ10*) should be taken into account. Of note, the high incidence of benign polymorphisms in the population could lead to false positive results. For this reason, genetic studies should be implemented together with functional studies, to unambiguously discriminate between pathogenic mutations and allelic variants that cannot be considered as genetic determinants for Pendred syndrome and ns-EVA. Assessing the functionality of pendrin allelic variants, the presence of hypo-functional variants and possible differences between the I^-/Cl^- and Cl^-/HCO_3^- exchange activities should be carefully evaluated.

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References

1 Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, Adawi F, Hazani E, Nassir E, Baxevanis AD, Sheffield VC, Green ED: Pendred syndrome is caused by mutations in a putative sulphate transporter gene (*PDS*). Nat Genet 1997;17:411-422.

2 Royaux IE, Suzuki K, Mori A, Katoh R, Everett LA, Kohn LD, Green ED: Pendrin, the protein encoded by the Pendred syndrome gene (*PDS*), is an apical porter of iodide in the thyroid and is regulated by thyroglobulin in FRTL-5 cells. Endocrinology 2000;141:839-845.

3 Royaux IE, Wall SM, Karniski LP, Everett LA, Suzuki K, Knepper MA, Green ED: Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion. Proc Natl Acad Sci U S A 2001;98:4221-4226.

4 Everett LA, Morsli H, Wu DK, Green ED: Expression pattern of the mouse ortholog of the Pendred's syndrome gene (*Pds*) suggests a key role for pendrin in the inner ear. Proc Natl Acad Sci USA 1999;96:9727-9732.

5 Royaux IE, Belyantseva IA, Wu T, Kachar B, Everett LA, Marcus DC, Green ED: Localization and functional studies of pendrin in the mouse inner ear provide insight about the etiology of deafness in pendred syndrome. J Assoc Res Otolaryngol 2003;4:394-404.

6 Pedemonte N, Caci E, Sondo E, Caputo A, Rhoden K, Pfeffer U, Di CM, Bandettini R, Ravazzolo R, Zegarra-Moran O, Galietta LJ: Thiocyanate transport in resting and IL-4-stimulated human bronchial epithelial cells: role of pendrin and anion channels. J Immunol 2007;178:5144-5153.

7 Rillema JA, Hill MA: Pendrin transporter carries out iodide uptake into MCF-7 human mammary cancer cells. Exp Biol Med (Maywood) 2003;228:1078-1082.

8 Lacroix L, Mian C, Caillou B, Talbot M, Filetti S, Schlumberger M, Bidart JM: Na^+/I^- symporter and Pendred syndrome gene and protein expressions in human extra-thyroidal tissues. Eur J Endocrinol 2001;144:297-302.

9 Bidart JM, Lacroix L, Evain-Brion D, Caillou B, Lazar V, Frydman R, Bellet D, Filetti S, Schlumberger M: Expression of Na^+/I^- symporter and Pendred syndrome genes in trophoblast cells. J Clin Endocrinol Metab 2000;85:4367-4372.

10 Suzuki K, Royaux IE, Everett LA, Mori-Aoki A, Suzuki S, Nakamura K, Sakai T, Katoh R, Toda S, Green ED, Kohn LD: Expression of *PDS/Pds*, the Pendred syndrome gene, in endometrium. J Clin Endocrinol Metab 2002;87:938.

11 Alesutan I, Daryadel A, Mohebbi N, Pelzl L, Leibrock C, Voelkl J, Bourgeois S, Dossena S, Nofziger C, Paulmichl M, Wagner CA, Lang F: Impact of bicarbonate, ammonium chloride and acetazolamide on hepatic and renal Slc26a4 expression. Cell Physiol Biochem 2011;28:553-558.

12 Kopp P, Pesce L, Solis-S JC: Pendred syndrome and iodide transport in the thyroid. Trends Endocrinol Metab 2008;19:260-268.

13 Dou H, Xu J, Wang Z, Smith AN, Soleimani M, Karet FE, Greinwald JH Jr, Choo D: Co-expression of pendrin, vacuolar H^+-ATPase alpha4-subunit and carbonic anhydrase II in epithelial cells of the murine endolymphatic sac. J Histochem Cytochem 2004;52:1377-1384.

14 Griffith AJ, Wangemann P: Hearing loss associated with enlargement of the vestibular aqueduct: Mechanistic insights from clinical phenotypes, genotypes, and mouse models. Hear Res 2011; in press.

15 Wangemann P, Itza EM, Albrecht B, Wu T, Jabba SV, Maganti RJ, Lee JH, Everett LA, Wall SM, Royaux IE, Green ED, Marcus DC: Loss of KCNJ10 protein expression abolishes endocochlear potential and causes deafness in Pendred syndrome mouse model. BMC Med 2004;2:30.

16 Yoshino T, Sato E, Nakashima T, Teranishi M, Yamamoto H, Otake H, Mizuno T: Distribution of pendrin in the organ of Corti of mice observed by electron immunomicroscopy. Eur Arch Otorhinolaryngol 2006;263:699-704.

17 Yoshino T, Sato E, Nakashima T, Nagashima W, Teranishi MA, Nakayama A, Mori N, Murakami H, Funahashi H, Imai T: The immunohistochemical analysis of pendrin in the mouse inner ear. Hear Res 2004;195:9-16.

18 Wangemann P, Nakaya K, Wu T, Maganti RJ, Itza EM, Sanneman JD, Harbidge DG, Billings S, Marcus DC: Loss of cochlear HCO3- secretion causes deafness via endolymphatic acidification and inhibition of Ca^{2+} reabsorption in a Pendred syndrome mouse model. Am J Physiol Renal Physiol 2007;292:F1345-F1353.

19 Wall SM, Hassell KA, Royaux IE, Green ED, Chang JY, Shipley GL, Verlander JW: Localization of pendrin in mouse kidney. Am J Physiol Renal Physiol 2003;284:F229-F241.

20 Kim YH, Kwon TH, Frische S, Kim J, Tisher CC, Madsen KM, Nielsen S: Immunocytochemical localization of pendrin in intercalated cell subtypes in rat and mouse kidney. Am J Physiol Renal Physiol 2002;283:F744-F754.
21 Amlal H, Petrovic S, Xu J, Wang Z, Sun X, Barone S, Soleimani M: Deletion of the anion exchanger Slc26a4 (pendrin) decreases apical Cl^-/HCO_3^- exchanger activity and impairs bicarbonate secretion in kidney collecting duct. Am J Physiol Cell Physiol 2010;299:C33-C41.
22 Eladari D, Chambrey R, Frische S, Vallet M, Edwards A: Pendrin as a regulator of ECF and blood pressure. Curr Opin Nephrol Hypertens 2009;18:356-362.
23 Wall SM, Pech V: Pendrin and sodium channels: relevance to hypertension. J Nephrol 2010;23:S118-S123.
24 Mount DB, Romero MF: The *SLC26* gene family of multifunctional anion exchangers. Pflugers Arch 2004;447:710-721.
25 Scott DA, Wang R, Kreman TM, Sheffield VC, Karniski LP: The Pendred syndrome gene encodes a chloride-iodide transport protein. Nat Genet 1999;21:440-443.
26 Bogazzi F, Bartalena L, Raggi F, Ultimieri F, Martino E: Pendrin does not increase sulfate uptake in mammalian COS-7 cells. J Endocrinol Invest 2000;23:170-172.
27 Kraiem Z, Heinrich R, Sadeh O, Shiloni E, Nassir E, Hazani E, Glaser B: Sulfate transport is not impaired in pendred syndrome thyrocytes. J Clin Endocrinol Metab 1999;84:2574-2576.
28 Yoshida A, Taniguchi S, Hisatome I, Royaux IE, Green ED, Kohn LD, Suzuki K: Pendrin is an iodide-specific apical porter responsible for iodide efflux from thyroid cells. J Clin Endocrinol Metab 2002;87:3356-3361.
29 Gillam MP, Sidhaye AR, Lee EJ, Rutishauser J, Stephan CW, Kopp P: Functional characterization of pendrin in a polarized cell system. Evidence for pendrin-mediated apical iodide efflux. J Biol Chem 2004;279:13004-13010.
30 Dossena S, Maccagni A, Vezzoli V, Bazzini C, Garavaglia ML, Meyer G, Furst J, Ritter M, Fugazzola L, Persani L, Zorowka P, Storelli C, Beck-Peccoz P, Botta G, Paulmichl M: The expression of wild-type pendrin (SLC26A4) in human embryonic kidney (HEK 293 Phoenix) cells leads to the activation of cationic currents. Eur J Endocrinol 2005;153:693-699.
31 Dossena S, Rodighiero S, Vezzoli V, Bazzini C, Sironi C, Meyer G, Furst J, Ritter M, Garavaglia ML, Fugazzola L, Persani L, Zorowka P, Storelli C, Beck-Peccoz P, Botta G, Paulmichl M: Fast fluorometric method for measuring pendrin (SLC26A4) Cl^-/I^- transport activity. Cell Physiol Biochem 2006;28:18:67-74.
32 Dossena S, Vezzoli V, Cerutti N, Bazzini C, Tosco M, Sironi C, Rodighiero S, Meyer G, Fascio U, Furst J, Ritter M, Fugazzola L, Persani L, Zorowka P, Storelli C, Beck-Peccoz P, Botta G, Paulmichl M: Functional characterization of wild-type and a mutated form of SLC26A4 identified in a patient with Pendred syndrome. Cell Physiol Biochem 2006;17:245-256.
33 Shcheynikov N, Yang D, Wang Y, Zeng W, Karniski LP, So I, Wall SM, Muallem S: The Slc26a4 transporter functions as an electroneutral $Cl^-/I^-/HCO_3^-$ exchanger: role of Slc26a4 and Slc26a6 in I^- and HCO_3^- secretion and in regulation of CFTR in the parotid duct. J Physiol 2008;586:3813-3824.
34 Wangemann P, Kim HM, Billings S, Nakaya K, Li X, Singh R, Sharlin DS, Forrest D, Marcus DC, Fong P: Developmental delays consistent with cochlear hypothyroidism contribute to failure to develop hearing in mice lacking Slc26a4/pendrin expression. Am J Physiol Renal Physiol 2009;297:F1435-F1447.
35 Twyffels L, Massart C, Golstein PE, Raspe E, Van Sande J, Dumont JE, Beauwens R, Kruys, V: Pendrin: the thyrocyte apical membrane iodide transporter? Cell Physiol Biochem 2011;28:491-496.
36 Bizhanova A, Kopp P: Controversies concerning the role of pendrin as apical iodide transporter in thyroid follicular cells. Cell Physiol Biochem 2011;28:485-490.
37 Soleimani M, Greeley T, Petrovic S, Wang Z, Amlal H, Kopp P, Burnham CE: Pendrin: an apical $Cl^-/OH^-/HCO_3^-$ exchanger in the kidney cortex. Am J Physiol Renal Physiol 2001;280:F356-F364.
38 Wagner CA, Finberg KE, Stehberger PA, Lifton RP, Giebisch GH, Aronson PS, Geibel JP: Regulation of the expression of the Cl^-/anion exchanger pendrin in mouse kidney by acid-base status. Kidney Int 2002;62:2109-2117.
39 Frische S, Kwon TH, Frokiaer J, Madsen KM, Nielsen S: Regulated expression of pendrin in rat kidney in response to chronic NH4Cl or NaHCO3 loading. Am J Physiol Renal Physiol 2003;284:F584-F593.
40 Petrovic S, Wang Z, Ma L, Soleimani M: Regulation of the apical Cl^-/HCO_3^- exchanger pendrin in rat cortical collecting duct in metabolic acidosis. Am J Physiol Renal Physiol 2003;284:F103-F112.
41 Nowik M, Kampik NB, Mihailova M, Eladari D, Wagner CA: Induction of metabolic acidosis with ammonium chloride (NH_4Cl) in mice and rats-species differences and technical considerations. Cell Physiol Biochem 2010;26:1059-1072.
42 Purkerson JM, Tsuruoka S, Suter DZ, Nakamori A, Schwartz GJ: Adaptation to metabolic acidosis and its recovery are associated with changes in anion exchanger distribution and expression in the cortical collecting duct. Kidney Int 2010;78:993-1005.
43 Bizhanova A, Kopp P: Genetics and phenomics of Pendred syndrome. Mol Cell Endocrinol 2010;322:83-90.
44 Kandasamy N, Fugazzola L, Evans M, Chatterjee K, Karet F: Life-threatening metabolic alkalosis in Pendred syndrome. Eur J Endocrinol 2011;165:167-170.
45 Pela I, Bigozzi M, Bianchi B: Profound hypokalemia and hypochloremic metabolic alkalosis during thiazide therapy in a child with Pendred syndrome. Clin Nephrol 2008;69:450-453.
46 Quentin F, Chambrey R, Trinh-Trang-Tan MM, Fysekidis M, Cambillau M, Paillard M, Aronson PS, Eladari D: The Cl^-/HCO_3^- exchanger pendrin in the rat kidney is regulated in response to chronic alterations in chloride balance. Am J Physiol Renal Physiol 2004;287:F1179-F1188.
47 Wall SM, Kim YH, Stanley L, Glapion DM, Everett LA, Green ED, Verlander JW: NaCl restriction upregulates renal Slc26a4 through subcellular redistribution: role in Cl- conservation. Hypertension 2004;44:982-987.
48 Verlander JW, Kim YH, Shin W, Pham TD, Hassell KA, Beierwaltes WH, Green ED, Everett L, Matthews SW, Wall SM: Dietary Cl^- restriction upregulates pendrin expression within the apical plasma membrane of type B intercalated cells. Am J Physiol Renal Physiol 2006;291:F833-F839.
49 Vallet M, Picard N, Loffing-Cueni D, Fysekidis M, Bloch-Faure M, Deschenes G, Breton S, Meneton P, Loffing J, Aronson PS, Chambrey R, Eladari D: Pendrin regulation in mouse kidney primarily is chloride-dependent. J Am Soc Nephrol 2006;17:2153-2163.
50 Kim YH, Pham TD, Zheng W, Hong S, Baylis C, Pech V, Beierwaltes WH, Farley DB, Braverman LE, Verlander JW, Wall SM: Role of pendrin in iodide balance: going with the flow. Am J Physiol Renal Physiol 2009;297:F1069-F1079.
51 Everett LA, Belyantseva IA, Noben-Trauth K, Cantos R, Chen A, Thakkar SI, Hoogstraten-Miller SL, Kachar B, Wu DK, Green ED: Targeted disruption of mouse Pds provides insight about the inner-ear defects encountered in Pendred syndrome. Hum Mol Genet 2001;10:153-161.
52 Nakaya K, Harbidge DG, Wangemann P, Schultz BD, Green ED, Wall SM, Marcus DC: Lack of pendrin HCO_3^- transport elevates vestibular endolymphatic $[Ca^{2+}]$ by inhibition of acid-sensitive TRPV5 and TRPV6 channels. Am J Physiol Renal Physiol 2007;292:F1314-F1321.

53 Kim HM, Wangemann P: Failure of fluid absorption in the endolymphatic sac initiates cochlear enlargement that leads to deafness in mice lacking pendrin expression. PLoS One 2010;5:e14041.
54 Dror AA, Politi Y, Shahin H, Lenz DR, Dossena S, Nofziger C, Fuchs H, Hrabe de AM, Paulmichl M, Weiner S, Avraham KB: Calcium oxalate stone formation in the inner ear as a result of an *Slc26a4* mutation. J Biol Chem 2010;285:21724-21735.
55 Kuperman DA, Lewis CC, Woodruff PG, Rodriguez MW, Yang YH, Dolganov GM, Fahy JV, Erle DJ: Dissecting asthma using focused transgenic modeling and functional genomics. J Allergy Clin Immunol 2005;116:305-311.
56 Nakao I, Kanaji S, Ohta S, Matsushita H, Arima K, Yuyama N, Yamaya M, Nakayama K, Kubo H, Watanabe M, Sagara H, Sugiyama K, Tanaka H, Toda S, Hayashi H, Inoue H, Hoshino T, Shiraki A, Inoue M, Suzuki K, Aizawa H, Okinami S, Nagai H, Hasegawa M, Fukuda T, Green ED, Izuhara K: Identification of pendrin as a common mediator for mucus production in bronchial asthma and chronic obstructive pulmonary disease. J Immunol 2008;180:6262-6269.
57 Nakagami Y, Favoreto S Jr, Zhen G, Park SW, Nguyenvu LT, Kuperman DA, Dolganov GM, Huang X, Boushey HA, Avila PC, Erle DJ: The epithelial anion transporter pendrin is induced by allergy and rhinovirus infection, regulates airway surface liquid, and increases airway reactivity and inflammation in an asthma model. J Immunol 2008;181:2203-2210.
58 Nofziger C, Dossena S, Suzuki S, Izuhara K, Paulmichl M: Pendrin function in Airway Epithelia. Cell Physiol Biochem 2011;28:571-578.
59 Cremers FP: Genetic causes of hearing loss. Curr Opin Neurol 1998;11:11-16.
60 Coyle B, Reardon W, Herbrick JA, Tsui LC, Gausden E, Lee J, Coffey R, Grueters A, Grossman4 A, Phelps PD, Luxon L, Kendall-Taylor P, Scherer SW, Trembath RC: Molecular analysis of the *PDS* gene in Pendred syndrome. Hum Mol Genet 1998;7:1105-1112.
61 Pendred V: Deaf-mutism and goitre. Lancet 1896;148:532.
62 Morgans ME, Trotter WR: Association of congenital deafness with goitre; the nature of the thyroid defect. Lancet 1958;1:607-609.
63 Colvin IB, Beale T, Harrop-Griffiths K: Long-term follow-up of hearing loss in children and young adults with enlarged vestibular aqueducts: relationship to radiologic findings and Pendred syndrome diagnosis. Laryngoscope 2006;116:2027-2036.
64 Phelps PD, Coffey RA, Trembath RC, Luxon LM, Grossman AB, Britton KE, Kendall-Taylor P, Graham JM, Cadge BC, Stephens SG, Pembrey ME, Reardon W: Radiological malformations of the ear in Pendred syndrome. Clin Radiol 1998;53:268-273.
65 Pryor SP, Madeo AC, Reynolds JC, Sarlis NJ, Arnos KS, Nance WE, Yang Y, Zalewski CK, Brewer CC, Butman JA, Griffith AJ: SLC26A4/PDS genotype-phenotype correlation in hearing loss with enlargement of the vestibular aqueduct (EVA): evidence that Pendred syndrome and non-syndromic EVA are distinct clinical and genetic entities. J Med Genet 2005;42:159-165.
66 Park HJ, Lee SJ, Jin HS, Lee JO, Go SH, Jang HS, Moon SK, Lee SC, Chun YM, Lee HK, Choi JY, Jung SC, Griffith AJ, Koo SK: Genetic basis of hearing loss associated with enlarged vestibular aqueducts in Koreans. Clin Genet 2005;67:160-165.
67 Azaiez H, Yang T, Prasad S, Sorensen JL, Nishimura CJ, Kimberling WJ, Smith RJ: Genotype-phenotype correlations for SLC26A4-related deafness. Hum Genet 2007;122:451-457.
68 Albert S, Blons H, Jonard L, Feldmann D, Chauvin P, Loundon N, Sergent-Allaoui A, Houang M, Joannard A, Schmerber S, Delobel B, Leman J, Journel H, Catros H, Dollfus H, Eliot MM, David A, Calais C, Drouin-Garraud V, Obstoy MF, Tran Ba HP, Lacombe D, Duriez F, Francannet C, Bitoun P, Petit C, Garabedian EN, Couderc R, Marlin S, Denoyelle F: *SLC26A4* gene is frequently involved in nonsyndromic hearing impairment with enlarged vestibular aqueduct in Caucasian populations. Eur J Hum Genet 2006;14:773-779.
69 Tsukamoto K, Suzuki H, Harada D, Namba A, Abe S, Usami S: Distribution and frequencies of *PDS* (*SLC26A4*) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: a unique spectrum of mutations in Japanese. Eur J Hum Genet 2003;11:916-922.
70 Fugazzola L, Cirello V, Dossena S, Rodighiero S, Muzza M, Castorina P, Lalatta F, Ambrosetti U, Beck-Peccoz P, Botta G, Paulmichl M: High phenotypic intrafamilial variability in patients with Pendred syndrome and a novel duplication in the *SLC26A4* gene: clinical characterization and functional studies of the mutated SLC26A4 protein. Eur J Endocrinol 2007;157:331-338.
71 Billerbeck AE, Cavaliere H, Goldberg AC, Kalil J, Medeiros-Neto G: Clinical and molecular genetics studies in Pendred's syndrome. Thyroid 1994;4:279-284.
72 Reardon W, Coffey R, Chowdhury T, Grossman A, Jan H, Britton K, Kendall-Taylor P, Trembath R: Prevalence, age of onset, and natural history of thyroid disease in Pendred syndrome. J Med Genet 1999;36:595-598.
73 Yang T, Vidarsson H, Rodrigo-Blomqvist S, Rosengren SS, Enerback S, Smith RJ: Transcriptional control of *SLC26A4* is involved in Pendred syndrome and nonsyndromic enlargement of vestibular aqueduct (DFNB4). Am J Hum Genet 2007;80:1055-1063.
74 Yang T, Gurrola JG, Wu H, Chiu SM, Wangemann P, Snyder PM, Smith RJ: Mutations of *KCNJ10* together with mutations of *SLC26A4* cause digenic nonsyndromic hearing loss associated with enlarged vestibular aqueduct syndrome. Am J Hum Genet 2009;84:651-657.
75 Verlander JW, Hassell KA, Royaux IE, Glapion DM, Wang ME, Everett LA, Green ED, Wall SM: Deoxycorticosterone upregulates PDS (Slc26a4) in mouse kidney: role of pendrin in mineralocorticoid-induced hypertension. Hypertension 2003;42:356-362.
76 Pech V, Kim YH, Weinstein AM, Everett LA, Pham TD, Wall SM: Angiotensin II increases chloride absorption in the cortical collecting duct in mice through a pendrin-dependent mechanism. Am J Physiol Renal Physiol 2007;292:F914-F920.
77 Nofziger C, Vezzoli V, Dossena S, Schonherr T, Studnicka J, Nofziger J, Vanoni S, Stephan S, Silva M, Meyer G, Paulmichl M: STAT6 Links IL-4/IL-13 Stimulation With Pendrin Expression in Asthma and Chronic Obstructive Pulmonary Disease. Clin Pharmacol Ther 2011;90:399-405.
78 Di Valentin E, Crahay C, Garbacki N, Hennuy B, Gueders M, Noel A, Foidart JM, Grooten J, Colige A, Piette J, Cataldo D: New asthma biomarkers: lessons from murine models of acute and chronic asthma. Am J Physiol Lung Cell Mol Physiol 2009;296:L185-L197.
79 Dossena S, Bizhanova A, Nofziger C, Bernardinelli E, Ramsauer J, Kopp P, Paulmichl M: Identification of allelic variants of pendrin (SLC26A4) with loss and gain of function. Cell Physiol Biochem 2011;28:467-476.
80 Salhany JM: Mechanism of competition between chloride and stilbenedisulfonates for binding to human erythrocyte band 3 (AE1). Biochem Cell Biol 1998;76:715-722.
81 Scott DA, Wang R, Kreman TM, Andrews M, McDonald JM, Bishop JR, Smith RJ, Karniski LP, Sheffield VC: Functional differences of the *PDS* gene product are associated with phenotypic variation in patients with Pendred syndrome and non-syndromic hearing loss (DFNB4). Hum Mol Genet 2000;9:1709-1715.

82 Taylor JP, Metcalfe RA, Watson PF, Weetman AP, Trembath RC: Mutations of the *PDS* gene, encoding pendrin, are associated with protein mislocalization and loss of iodide efflux: implications for thyroid dysfunction in Pendred syndrome. J Clin Endocrinol Metab 2002;87:1778-1784.
83 Pfarr N, Borck G, Turk A, Napiontek U, Keilmann A, Muller-Forell W, Kopp P, Pohlenz J: Goitrous congenital hypothyroidism and hearing impairment associated with mutations in the *TPO* and *SLC26A4/PDS* genes. J Clin Endocrinol Metab 2006;91:2678-2681.
84 Gillam MP, Bartolone L, Kopp P, Benvenga S: Molecular analysis of the *PDS* gene in a nonconsanguineous Sicilian family with Pendred's syndrome. Thyroid 2005;15:734-741.
85 Ishihara K, Okuyama S, Kumano S, Iida K, Hamana H, Murakoshi M, Kobayashi T, Usami S, Ikeda K, Haga Y, Tsumoto K, Nakamura H, Hirasawa N, Wada H: Salicylate restores transport function and anion exchanger activity of missense pendrin mutations. Hear Res 2010;270:110-118.
86 Pera A, Dossena S, Rodighiero S, Gandia M, Botta G, Meyer G, Moreno F, Nofziger C, Hernandez-Chico C, Paulmichl M: Functional assessment of allelic variants in the *SLC26A4* gene involved in Pendred syndrome and nonsyndromic EVA. Proc Natl Acad Sci USA 2008;105:18608-18613.
87 Galietta LJ, Haggie PM, Verkman AS: Green fluorescent protein-based halide indicators with improved chloride and iodide affinities. FEBS Lett 2001;499:220-224.
88 Yoon JS, Park HJ, Yoo SY, Namkung W, Jo MJ, Koo SK, Park HY, Lee WS, Kim KH, Lee MG: Heterogeneity in the processing defect of SLC26A4 mutants. J Med Genet 2008;45:411-419.
89 Choi BY, Stewart AK, Madeo AC, Pryor SP, Lenhard S, Kittles R, Eisenman D, Kim HJ, Niparko J, Thomsen J, Arnos KS, Nance WE, King KA, Zalewski CK, Brewer CC, Shawker T, Reynolds JC, Butman JA, Karniski LP, Alper SL, Griffith AJ: Hypo-functional SLC26A4 variants associated with nonsyndromic hearing loss and enlargement of the vestibular aqueduct: genotype-phenotype correlation or coincidental polymorphisms? Hum Mutat 2009;30:599-608.
90 Dai P, Stewart AK, Chebib F, Hsu A, Rozenfeld J, Huang D, Kang D, Lip V, Fang H, Shao H, Liu X, Yu F, Yuan H, Kenna M, Miller DT, Shen Y, Yang W, Zelikovic I, Platt OS, Han D, Alper SL, Wu BL: Distinct and novel SLC26A4/Pendrin mutations in Chinese and U.S. patients with nonsyndromic hearing loss. Physiol Genomics 2009;38:281-290.
91 Dossena S, Nofziger C, Brownstein ZN, Kanaan M, Avraham KB, Paulmichl M: Functional characterization of Pendrin mutations found in the Israeli and Palestinian populations. Cell Physiol Biochem 2011;28:477-484.
92 Palos F, Garcia-Rendueles ME, raujo-Vilar D, Obregon MJ, Calvo RM, Cameselle-Teijeiro J, Bravo SB, Perez-Guerra O, Loidi L, Czarnocka B, Alvarez P, Refetoff S, Dominguez-Gerpe L, Alvarez CV, Lado-Abeal J: Pendred syndrome in two Galician families: insights into clinical phenotypes through cellular, genetic, and molecular studies. J Clin Endocrinol Metab 2008;93:267-277.
93 Dossena S, Rodighiero S, Vezzoli V, Nofziger C, Salvioni E, Boccazzi M, Grabmayer E, Botta G, Meyer G, Fugazzola L, Beck-Peccoz P, Paulmichl M: Functional characterization of wild-type and mutated pendrin (SLC26A4), the anion transporter involved in Pendred syndrome. J Mol Endocrinol 2009;43:93-103.
94 Brownstein ZN, Dror AA, Gilony D, Migirov L, Hirschberg K, Avraham KB: A novel *SLC26A4* (*PDS*) deafness mutation retained in the endoplasmic reticulum. Arch Otolaryngol Head Neck Surg 2008;134:403-407.
95 Walsh T, Abu RA, Abu SJ, Shahin H, Shepshelovich J, Lee MK, Hirschberg K, Tekin M, Salhab W, Avraham KB, King MC, Kanaan M: Genomic analysis of a heterogeneous Mendelian phenotype: multiple novel alleles for inherited hearing loss in the Palestinian population. Hum Genomics 2006;2:203-211.
96 Rotman-Pikielny P, Hirschberg K, Maruvada P, Suzuki K, Royaux IE, Green ED, Kohn LD, Lippincott-Schwartz J, Yen PM: Retention of pendrin in the endoplasmic reticulum is a major mechanism for Pendred syndrome. Hum Mol Genet 2002;11:2625-2633.
97 Park HJ, Shaukat S, Liu XZ, Hahn SH, Naz S, Ghosh M, Kim HN, Moon SK, Abe S, Tukamoto K, Riazuddin S, Kabra M, Erdenetungalag R, Radnaabazar J, Khan S, Pandya A, Usami SI, Nance WE, Wilcox ER, Riazuddin S, Griffith AJ: Origins and frequencies of *SLC26A4* (*PDS*) mutations in east and south Asians: global implications for the epidemiology of deafness. J Med Genet 2003;40:242-248.
98 Shepshelovich J, Goldstein-Magal L, Globerson A, Yen PM, Rotman-Pikielny P, Hirschberg K: Protein synthesis inhibitors and the chemical chaperone TMAO reverse endoplasmic reticulum perturbation induced by overexpression of the iodide transporter pendrin. J Cell Sci 2005;118:1577-1586.
99 Kopp P, Bizhanova A: Clinical and molecular characteristics of Pendred syndrome. Ann Endocrinol (Paris) 2011;72:88-94.
100 Banghova K, Al TE, Novotna D, Zapletalova J, Hnikova O, Cap J, Klabochova J, Kusekova M, Lebl J: Pendred syndrome among patients with hypothyroidism: genetic diagnosis, phenotypic variability and occurrence of phenocopies. Cas Lek Cesk 2008;147:616-622.
101 Fugazzola L, Cerutti N, Mannavola D, Crino A, Cassio A, Gasparoni P, Vannucchi G, Beck-Peccoz P: Differential diagnosis between Pendred and pseudo-Pendred syndromes: clinical, radiologic, and molecular studies. Pediatr Res 2002;51:479-484.
102 Kopp P, Arseven OK, Sabacan L, Kotlar T, Dupuis J, Cavaliere H, Santos CL, Jameson JL, Medeiros-Neto G: Phenocopies for deafness and goiter development in a large inbred Brazilian kindred with Pendred's syndrome associated with a novel mutation in the *PDS* gene. J Clin Endocrinol Metab 1999;84:336-341.
103 Fugazzola L, Mannavola D, Cerutti N, Maghnie M, Pagella F, Bianchi P, Weber G, Persani L, Beck-Peccoz P: Molecular analysis of the Pendred's syndrome gene and magnetic resonance imaging studies of the inner ear are essential for the diagnosis of true Pendred's syndrome. J Clin Endocrinol Metab 2000;85:2469-2475.
104 Rebeh IB, Yoshimi N, Hadj-Kacem H, Yanohco S, Hammami B, Mnif M, Araki M, Ghorbel A, Ayadi H, Masmoudi S, Miyazaki H: Two missense mutations in *SLC26A4* gene: a molecular and functional study. Clin Genet 2010;78:74-80.