

## REVIEW

# Functional characterization of wild-type and mutated pendrin (SLC26A4), the anion transporter involved in Pendred syndrome

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## Abstract

Pendred syndrome (PS) is the most frequent form of genetically related syndromic hearing loss, and is associated with mutations of pendrin, encoded by the *SLC26A4* gene. This protein localizes to the cellular membrane and permits the exchange of anions between the cytosol and extracellular space. In the inner ear, pendrin conditions the endolymph, allowing for the proper function of sensory cells. Understanding the relationship between the genotype and phenotype of pendrin mutations would aid clinicians to better serve PS patients—however, little is known. Here, we summarize the available data concerning *SLC26A4* mutations and how they relate to transporter function. The main findings suggest that all the truncation mutations tested annihilate pendrin function, and that the addition or omission of proline, or the addition or omission of charged amino acids in the sequence of *SLC26A4* result in a substantial to dramatic reduction in pendrin function.

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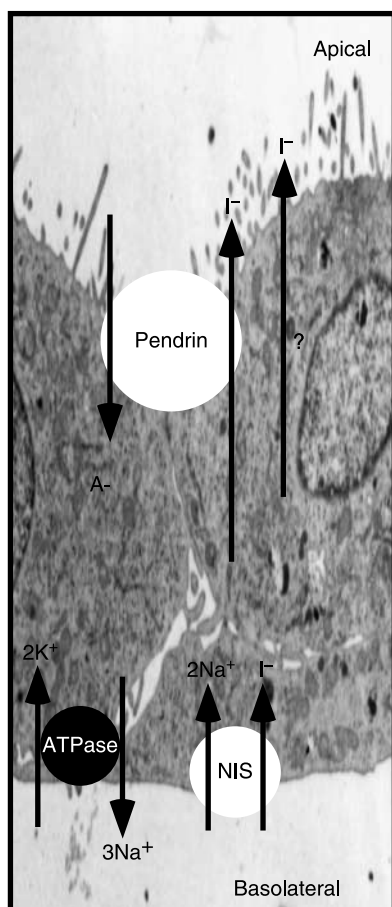
## Pendrin function

Pendrin is a member of the anion transporter family SLC26, which mediates, as shown in Fig. 1, the exchange of anions including  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ,  $\text{OH}^-$ ,  $\text{I}^-$ , or formate (Mount & Romero 2004), and is encoded by the *SLC26A4* gene. As shown in Fig. 1, in thyrocytes, iodide and sodium are brought into the cells via the basolaterally located sodium–iodide symporter. Apically located pendrin (Royaux *et al.* 2000) seems to be responsible for the efflux of iodide into the follicular lumen (Yoshida *et al.* 2002, 2004, Gillam *et al.* 2004). In the kidney, pendrin is suspected to mediate  $\text{Cl}^-/\text{HCO}_3^-$  exchange in the acid–base regulating  $\beta$ - and non- $\alpha$ -non- $\beta$ -intercalated cells (Royaux *et al.* 2001, Soleimani *et al.* 2001, Kim *et al.* 2002). A substantial body of work is supporting this concept (Wall 2005, Cantone *et al.* 2006, Soleimani & Xu 2006, Grimaldi *et al.* 2007, Hughey & Kleyman 2007, Sindic *et al.* 2007, Wall & Pech 2008). Similarly, in the inner ear, pendrin is thought to

mediate  $\text{Cl}^-/\text{HCO}_3^-$  exchange, and is therefore involved in the conditioning of endolymphatic fluid, presumably due to  $\text{HCO}_3^-$  secretion (Wangemann *et al.* 2007). Malfunction of pendrin leads to Pendred syndrome (PS).

## Pendred syndrome: clinical aspects

PS (OMIM#274600) is an autosomal recessive disorder accounting for 4–10% of inherited hearing losses (Batsakis & Nishiyama 1962, Fraser 1965, Illum *et al.* 1972, Reardon *et al.* 1997). It was first described in 1896 as the combination of deafness and goiter (Pendred 1896), but the precise phenotype has been detailed in recent years (Masmoudi *et al.* 2000, Campbell *et al.* 2001, Blons *et al.* 2004, Napiontek *et al.* 2004) and involves two organ systems: the ear, and the thyroid gland. It is unclear whether or not the kidney could be involved under certain circumstances as well.



**Figure 1** Cell model of the ion transport mechanisms in follicular cells of the thyroid gland. The sodium/potassium ATPase and the sodium/iodide symporter (NIS) are located on the basolateral side of follicular cells. At the apical side, pendrin is responsible for iodide transport into the follicles. However, an additional unknown iodide transporter may be expressed in the apical membrane.

### SLC26A4 mutations: the ear

Under normal conditions, pendrin maintains the ionic composition of endolymph (Everett *et al.* 1999). It has therefore been hypothesized that impaired pendrin function i) promotes a progressive increase in endolymph volume followed by an enlargement of the membranous labyrinth and surrounding osseous structures, and ii) leads to degeneration of inner ear sensory cells (Everett *et al.* 2001). The resulting phenotype is a severe/profound sensorineural hearing loss (SNHL). The onset of deafness fluctuates in about 80% of cases. By contrast, the sudden development of the phenotype occurs only in a minority of patients. SNHL is invariably associated with malformations of the inner ear: enlarged vestibular aqueduct (EVA) is present in all patients with PS (Phelps *et al.* 1998), whereas Mondini malformations are less common

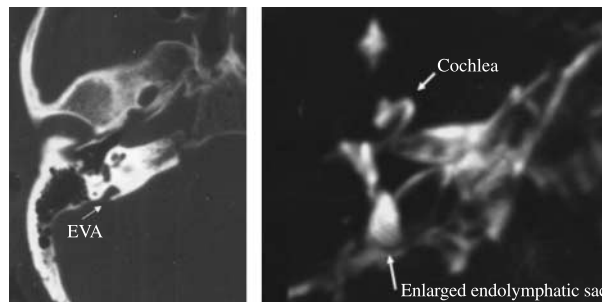
(Yang *et al.* 2005). These abnormalities can be detected in PS patients by computed tomography or nuclear-magnetic resonance of the petrous part of the temporal bone (Fig. 2).

### SLC26A4 mutations: the thyroid

Impaired pendrin function at the thyroid level can result in goiter, defects in iodide organification, and hypothyroidism (Fugazzola *et al.* 2001, Grimaldi *et al.* 2007, Kopp *et al.* 2008). Surprisingly, the thyroid symptoms are highly variable. Indeed, goiter is not a constant feature and can range from a slight increase in thyroid size to a large multinodular goiter. Perchlorate tests have shown that the organification defect is only partial (Fugazzola *et al.* 2000), indicating the existence of another mechanism underlying the transfer of iodine from the cytoplasm to the colloid (Fig. 1), such as ion channels (Golstein *et al.* 1992, Yoshida *et al.* 1999). Accordingly, most patients are euthyroid or subclinical hypothyroid, depending on the level of iodine intake.

### SLC26A4 mutations: the kidney

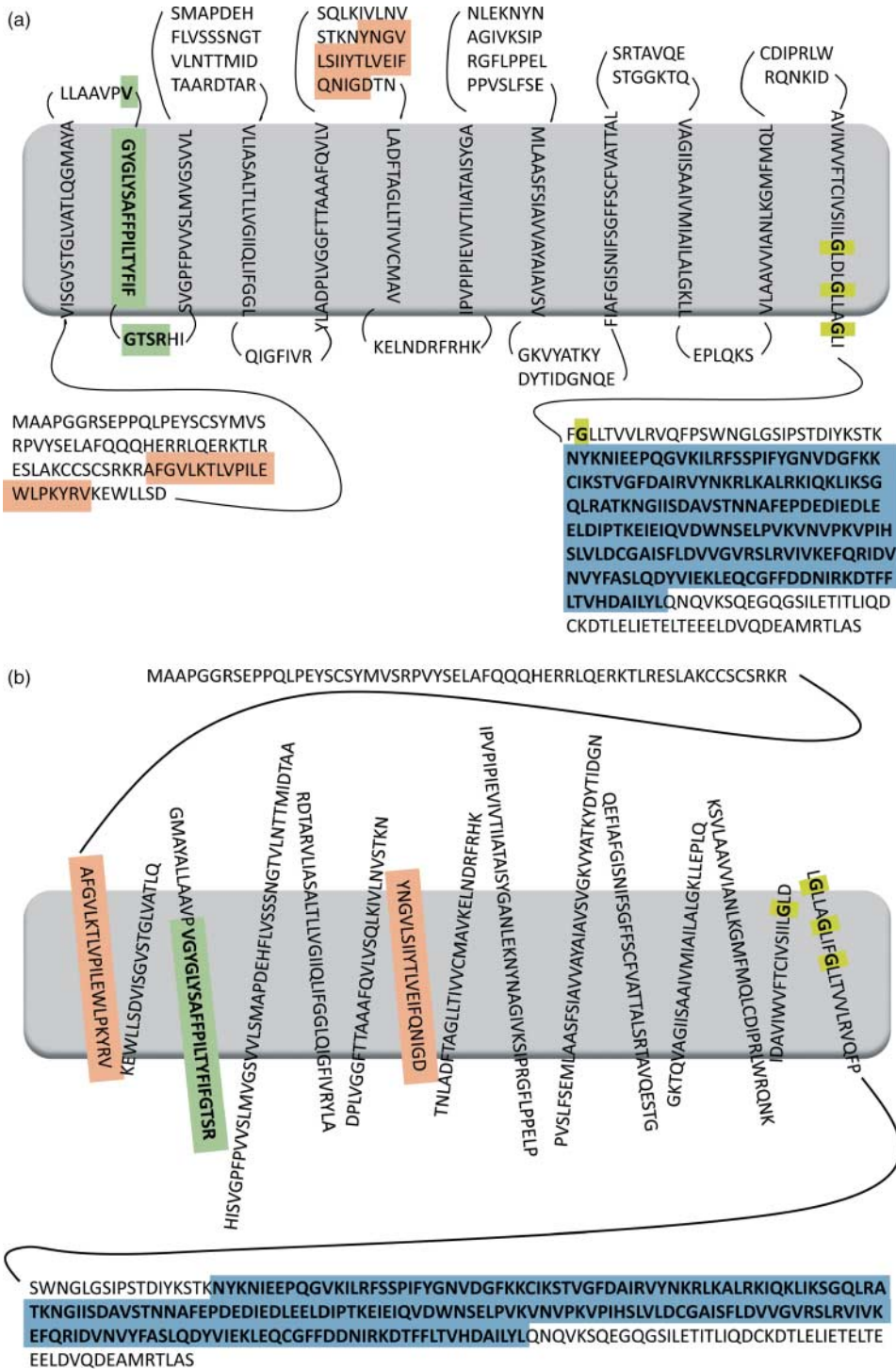
As far as the kidney is concerned, a decrease in pendrin function is not associated with disturbances in renal function. In particular, the regulation of electrolytes and acid–base balance remains normal despite the critical role of pendrin in bicarbonate secretion (Royaux *et al.* 2001). Indeed, when studied under basal conditions, no renal abnormalities have been reported in either PS patients or *Pds*-knockout animals. It is assumed that in the kidney, pendrin-dependent ion transport is safeguarded by redundant mechanisms, which most likely attenuate the change in intracellular and systemic pH expected to result from pendrin impairment (Kim *et al.* 2005). However, differences become apparent under conditions wherein the transporter is stimulated.



**Figure 2** Enlarged vestibular aqueduct (EVA) and enlarged endolymphatic sac in PS patients. Computed tomographic (CT)-image (left), and a magnetic resonance image (MRI; right) of the temporal bone of two PS patients with EVA are indicated by an arrow in the CT-image, while the MRI shows an enlarged endolymphatic sac.



**Figure 3** Sequence comparison of SLC26A4 in different species. (1) *Homo sapiens* (NP\_000432), (2) *Rattus norvegicus* (NP\_062087), (3) *Mus musculus* (NP\_035997), (4) *Pan troglodytes* (XP\_519308), (5) *Macaca mulatta* (XP\_001094049), (6) *Canis familiaris* (XP\_540382), (7) *Bos taurus* (XP\_608706), (8) *Monodelphis domestica* (XP\_001363598), (9) *Gallus gallus* (XP\_425419), (10) *Danio rerio* (XP\_692273), and (11) *Xenopus laevis* (NP\_001089008). The 15 putative trans-membrane (TM) helices are indicated with arrows and respective numbering (S1–S15). The two amphipathic helices are boxed in red, and the STAS-domain is boxed in blue. The sulfate-transport-consensus-signature is boxed in green, and the glycine repeat is indicated in yellow.



**Figure 4** Putative topology of human pendrin. (a) The 12 TM model as described on the Pendred/BOR homepage (<http://www.healthcare.uiowa.edu/labs/pendredandbor/domains.htm>) using the MEMSAT program. (b) The 15 TM-segment model as proposed in this review. The amino terminus in this model would be located on the extracellular side, and the carboxy terminus would be located at the cytoplasmic side. The 15 trans-membrane (TM) segments are depicted as stretches of amino acids crossing the membrane, which is shown in grey. The position of the TM helices with respect to the membrane (the part of the putative helix that is exposed to the lipid moiety of the membrane) is tentative. The color code of the boxes is equal to that in Fig. 3. It is important to mention that both models are speculative; however, the two models depicted here will help to design experiments, which are needed in order to define the real nature and position of the different TM units.

**Table 1** Sequence of the putative 15 trans-membrane segments, as well as the amino terminal and the carboxy terminal end of human pendrin

	First aa number	Sequence
<b>Segment</b>		
Amino terminal	1	MAAPGGRSEPPQLPEYSCSYMVSRRPVYSELAFQQQHERRLQERKTLRESLAKCCSCSRKR
S1	61	AFGLVLTLPVILEWLPKYRV (amphipathic)
S2	81	KEWLLSDVISGVSTGLVATLQ
S3	102	GMAYALLAAVPVGYGLYSAFFPILTYFIFGTSR
S4	135	HISVGPFPVSLMVGSVVLSMAPDEHFLVSSNGTVLNTTMTIDTA
S5	181	RDTARVLIASALTLLVGIIQLIFGGLQIGFIVRYLA
S6	217	DPLVGGFTTAAAFQVLVSQLKIVLNVSTKN
S7	247	YNGVLSIIYTLVEIFQNIQD (amphipathic)
S8	267	TNLADFTAGLLTIVVCMVAVKELNDRFRHK
S9	296	IPVPIPIEVIVTIIATAISYGANLEKKNYNAGIVKSIPRGFLPPELP
S10	342	PVSLFSEMLAASFIAVVAYIAVSVGVKVVYATKYDYTIDGN
S11	383	QEFIAFGISNIFSGFFSCFVATTALSRTAVQUESTG
S12	418	GKTQVAGIISAAIVMIALALGKLEPLQ
S13	447	KSVLAAVVIANLKGFMFQLCDIPRLWRQNK
S14	477	IDAVIWFVTCIVSIIIGLD
S15	496	LGLLAGLIFGLLTVVLRVQFP
Carboxy terminal	517	SWNGLGSPSTDIYKSTKNYKNEEPQGVKILRFSSPIFYGNVDGFKKCIKSTVGFDAIRVYNK RLKALRKIQKLIKSGQLRATKNGIISDAVSTNNAFEPDEDIEDLEELDIPTKEIEIQVDWNSLPEL VKVNVPKVPIHSLVLDGSAISFLDVGVRSLRVVKEFQRIDVNVYFASLQDYVIEKLEQCGF FDDNIRKDTFFLTVHDAILYLQNVKSEQGQGSILETITLIQDCKDTLELIELTEEELDVQDEA MRTLAS

Following treatment with aldosterone analogs, weight gain, and hypertension are observed in SLC26A4+/+ but not in SLC26A4-/- mice (Wall 2006). Careful studies of renal function after basic and acid loading in PS patients should be performed, and could reveal abnormal handling of anions in the kidney.

No systematic genotype-phenotype correlations have been made so far in PS patients (Lopez-Bigas *et al.* 1999, Masmoudi *et al.* 2000, Fugazzola *et al.* 2007). This review is an attempt to summarize the functional data available as of now for pendrin and its mutants, and to correlate these data to the genotype identified, with the hope that this information will help clinicians to better treat PS patients.

## The molecular entity responsible for Pendred syndrome: structural aspects

The clinical patterns described at the level of the thyroid gland and the inner ear are the result of decreases in pendrin function (Everett *et al.* 1997), a protein encoded by the *SLC26A4* gene (NM\_000441). There is a high degree of similarity between pendrin in different species. Figure 3 shows the amino acid sequences of pendrin from *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Pan troglodytes*, *Macaca mulatta*, *Canis familiaris*, *Bos taurus*, *Monodelphis domestica*, *Gallus gallus*, *Danio rerio*, and *Xenopus laevis*. The respective similarities if compared with the human sequence

are 72.5%, with *P. troglodytes* and *M. mulatta* being 98.8% similar, and *R. norvegicus*, *M. musculus*, *C. familiaris*, and *M. domestica* being  $\geq 90\%$  similar (the similarity plot was done by ClustalW; MacVector). Structural information regarding membrane topology of pendrin is limited and controversial. Whereas Everett *et al.* (1997) suggest a 11 TM-segment model with the carboxy (C)-terminus at the extracellular site using the PHDhtm program, Royaux *et al.* (2000) suggest a 12 TM model, with the C-terminus at the cytoplasmic site. A similar 12 TM model is suggested on the Pendred/BOR homepage (<http://www.healthcare.uiowa.edu/labs/pendredandbor/domains.htm>) using the MEMSAT program (Zhai & Saier 2001; Fig. 4a). This program, however, predicts an additional 13th TM segment located in the sulfate-transporters-antisigma-factor-antagonist (STAS)-domain of SLC26A4 (Aravind & Koonin 2000), a sequence highly similar to the antisigma-factor-antagonist in bacteria. This sequence is believed to be involved in NTP binding and/or hydrolysis. Aravind & Koonin (2000) postulate that the STAS domain in SLC26-family members could possibly regulate anion-transport by sensing intracellular concentrations of GTP and/or ATP. Furthermore, it was hypothesized that the STAS domain is involved in the interaction of SLC26 members with the cystic-fibrosis-transmembrane-regulator (Ko *et al.* 2002, 2004); however, the exact function of this domain is still unclear. The 13th TM would bring the C-terminus toward the extracellular site and would therefore be in contrast to the experimental data provided by Gillam

**Table 2** Summary of all functionally characterized allelic variants of SLC26A4

	Pathology	Localization	Function	References
<b>Mutation</b>				
S28R	PS	PM <sup>a</sup>	Loss of chloride uptake <sup>b</sup> Loss of chloride and iodide transport <sup>c</sup>	Dossena <i>et al.</i> (2006a,b) and Yoon <i>et al.</i> (2008)
E29Q	EVA	Intracellular <sup>d</sup>	Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	
V88I	EVA		Reduction of the chloride and iodide transport <sup>c</sup>	Pera <i>et al.</i> (2008)
FS93>96X <sup>f</sup> + C416-1G <sub>A</sub> <sup>g</sup>	EVA (+ R409H) PS	Intracellular <sup>a,h</sup>	Chloride and iodide transport not reduced <sup>c</sup> Intracellular iodine retention (primary thyrocytes culture) <sup>i</sup>	Pera <i>et al.</i> (2008) Palos <i>et al.</i> (2008)
G102R	PS	ER <sup>j</sup>	Loss of iodide efflux <sup>k</sup>	Taylor <i>et al.</i> (2002)
L117F	EVA	PM <sup>j</sup>	Iodide efflux equal to wt <sup>k</sup>	Taylor <i>et al.</i> (2002)
V138F	PS	ER <sup>j</sup>	Loss of iodide efflux <sup>k</sup>	Taylor <i>et al.</i> (2002)
P140H	EVA		Loss of chloride and iodide transport <sup>c</sup>	Pera <i>et al.</i> (2008)
P142R	EVA	Intracellular <sup>d</sup>	Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	Yoon <i>et al.</i> (2008)
M147V	EVA	Intracellular <sup>d</sup>	Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	Yoon <i>et al.</i> (2008)
S166N	EVA	PM <sup>d</sup>	Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity not different from wt <sup>e</sup>	Yoon <i>et al.</i> (2008)
G209V	PS, EVA	PM <sup>j</sup>	Reduction of iodide efflux <sup>k</sup>	Taylor <i>et al.</i> (2002)
L236P	PS PS	ER <sup>j</sup> ER <sup>j</sup>	Loss of iodide efflux <sup>k</sup> Loss of chloride and iodide uptake <sup>b,i</sup>	Scott <i>et al.</i> (2000) Taylor <i>et al.</i> (2002)
		ER <sup>j</sup> Intracellular <sup>d</sup>		Rotman-Pikielny <i>et al.</i> (2002) Choi <i>et al.</i> (2008) and Yoon <i>et al.</i> (2008)
FS297>302X	PS, EVA		Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	
F335L	PS EVA	PM <sup>j</sup>	Loss of Cl <sup>-</sup> /I <sup>-</sup> exchange activity <sup>l</sup> Loss of iodide efflux <sup>k</sup> Reduction of Cl <sup>-</sup> /I <sup>-</sup> and Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>l</sup>	Gillam <i>et al.</i> (2005) Choi <i>et al.</i> (2008)
FS306>309X	PS	Intracellular <sup>j</sup>	Loss of iodide efflux <sup>k</sup>	Gillam <i>et al.</i> (2004)
E384G	PS	ER <sup>j</sup>	Loss of chloride and iodide uptake <sup>b,i</sup>	Scott <i>et al.</i> (2000) Rotman-Pikielny <i>et al.</i> (2002)
		Intracellular <sup>d</sup>	Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	Choi <i>et al.</i> (2008) and Yoon <i>et al.</i> (2008)
R409H	EVA PS	Partially PM <sup>a</sup>	Loss of Cl <sup>-</sup> /I <sup>-</sup> exchange activity <sup>l</sup> Loss of iodide efflux <sup>k</sup> Reduction of iodide and chloride transport <sup>c</sup>	Gillam <i>et al.</i> (2005) and Dossena S & Paulmichl M (personal communication)
V88I/R409H <sup>m</sup>	EVA		Reduction of the chloride and iodide transport <sup>c</sup>	Pera <i>et al.</i> (2008)
T410M	EVA	ER <sup>j</sup>	Loss of iodide efflux <sup>k</sup>	Taylor <i>et al.</i> (2002)
Q413P	PS		Loss of chloride and iodide transport <sup>c</sup>	Pera <i>et al.</i> (2008)
T416P	PS	ER <sup>j</sup>	Loss of chloride and iodide uptake <sup>b,i</sup>	Scott <i>et al.</i> (2000), Rotman-Pikielny <i>et al.</i> (2002)
		Intracellular <sup>d</sup>	Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	Choi <i>et al.</i> (2008) and Yoon <i>et al.</i> (2008)
G424D	PS		Loss of Cl <sup>-</sup> /I <sup>-</sup> exchange activity <sup>l</sup>	Yoon <i>et al.</i> (2008)
Q446R	EVA	ER <sup>j</sup>	Reduction of the chloride and iodide transport <sup>c</sup>	Pera <i>et al.</i> (2008)
V480D	EVA		Loss of iodide efflux <sup>k</sup>	Taylor <i>et al.</i> (2002)
T485R	PS		Reduction of chloride and iodide uptake <sup>b,i</sup>	Scott <i>et al.</i> (2000)
I490L	EVA		Reduction of the chloride and iodide transport <sup>c</sup>	Pera <i>et al.</i> (2008)
G497S	EVA	Intracellular <sup>d</sup>	Mild reduction of chloride and iodide uptake <sup>b,i</sup> Strong reduction of chloride and iodide uptake <sup>b,i</sup>	Scott <i>et al.</i> (2000) Scott <i>et al.</i> (2000) and Yoon <i>et al.</i> (2008)
		EVA	Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	
I490L+G497S <sup>n</sup>	EVA		Strong reduction of chloride and iodide uptake	Scott <i>et al.</i> (2000)
Q514K	EVA, PS		Loss of chloride and iodide transport <sup>c</sup>	Pera <i>et al.</i> (2008)
FS523>548X	PS		Loss of chloride and iodide transport <sup>c</sup>	Fugazzola <i>et al.</i> (2007)
Y556C	PS	Partially PM <sup>j</sup>	Loss of iodide efflux <sup>k</sup>	Taylor <i>et al.</i> (2002)
C565Y	EVA	PM <sup>j</sup>	Reduction of Cl <sup>-</sup> /I <sup>-</sup> and Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>l</sup>	Choi <i>et al.</i> (2008)
L597S	Controls only	PM <sup>j</sup>	Chloride and iodide transport not different from wt <sup>c</sup>	Choi <i>et al.</i> (2008) and Pera <i>et al.</i> (2008)
		EVA	Reduction of Cl <sup>-</sup> /I <sup>-</sup> and Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>l</sup>	
E625X	EVA	Intracellular <sup>d</sup>	Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	Yoon <i>et al.</i> (2008)
V653A	EVA		Reduction of chloride and iodide uptake <sup>b,i</sup>	Scott <i>et al.</i> (2000)

(continued)

Table 2 Continued

	Pathology	Localization	Function	References
<b>Mutation</b>				
G672E	PS	Partially PM <sup>l</sup>	Loss of iodide efflux <sup>k</sup>	Taylor <i>et al.</i> (2002)
L676Q	PS	Intracellular <sup>d</sup>	Loss of iodide efflux <sup>k</sup>	Gillam <i>et al.</i> (2004) and Yoon <i>et al.</i> (2008)
	PS?	Intracellular <sup>d</sup>	Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	Yoon <i>et al.</i> (2008)
H723R	PS	Intracellular <sup>d</sup>	Loss of Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>e</sup>	Yoon <i>et al.</i> (2008)
D724G	EVA		Loss of chloride and iodide transport <sup>c</sup>	Pera <i>et al.</i> (2008)
M775T	EVA	PM <sup>i</sup>	Reduction of Cl <sup>-</sup> /I <sup>-</sup> and Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>l</sup>	Choi <i>et al.</i> (2008)
R776C	TPO mut. EVA	PM <sup>i</sup>	Iodide efflux equal to wt <sup>k</sup> Reduction of Cl <sup>-</sup> /I <sup>-</sup> and Cl <sup>-</sup> /HCO <sub>3</sub> <sup>-</sup> exchange activity <sup>l</sup>	Pfarr <i>et al.</i> (2006) and Choi <i>et al.</i> (2008)

<sup>a</sup>Western blot.<sup>b</sup><sup>36</sup>Cl<sup>-</sup> uptake.<sup>c</sup>Fluorometric method.<sup>d</sup>N-glycosylation.<sup>e</sup>Measure of the pHi (BCECF).<sup>f</sup>Originally reported as: c.279delT.<sup>g</sup>Acceptor splice site mutation.<sup>h</sup>Confocal microscopy.<sup>i</sup><sup>125</sup>I<sup>-</sup> uptake.<sup>j</sup>GFP-fusion protein.<sup>k</sup><sup>125</sup>I<sup>-</sup> efflux.<sup>l</sup><sup>36</sup>Cl<sup>-</sup> efflux (rate constant evaluation).<sup>m</sup>Both mutations are present on the same molecule.<sup>n</sup>Mutations are present on different alleles.

*et al.* (2004), and therefore it was probably ignored in the 12 TM model using the MEMSAT program. Here, we show another putative model of SLC26A4, using the MEMSAT prediction as a starting point and after refining the model according Sweet & Eisenberg (1983), and Shafir & Guy (2004). In this model, SLC26A4 would be formed by up to 15 TM helical segments (Table 1 and Fig. 4b). The TM segments marked amphipathic are the most ambiguous in terms of their localization, which could be transmembrane, cytosolic, or extracellular. However, since the sequence homology of these segments between the different species is very high (Fig. 3), it could be assumed that these segments could indeed form TM helices as opposed to helices located on either surface of the membrane. However, more rigorous experiments are needed to distinguish between the different possibilities. By assuming that the C-terminus is located within the cytosol, the amino terminus would have to be located on the extracellular side in both the 15 TM and 13 TM (minus the two amphipathic helices) models. This is in direct contrast to the model suggested by Gillam, in which the amino terminus is located within the cytosol (Gillam *et al.* 2004). Again, all the SLC26A4 models proposed so far, including our model proposed here, are speculative and lack experimental evidence; therefore, more rigorous experiments are necessary to unambiguously determine the secondary and tertiary structure of SLC26A4. The third TM segment in Fig. 4b harbors the sulfate-transport-consensus-signature (Mount

& Romero 2004), a stretch of amino acids involved in the transport of sulfate in SLC26A1, A2, A3, A6-9, and A11 (Mount & Romero 2004). This sequence is, however, modified in SLC26A4, which is consistent with the data showing that pendrin does not transport sulfate (Scott *et al.* 1999, Bogazzi *et al.* 2000, Scott & Karniski 2000). An additional structural aspect of pendrin, as shown in Figs 3 and 4b, is the presence of glycines at every fourth position in the putative TM segment 15 (boxed in yellow in Figs 3 and 4b). It is possible that the arrangement of these amino acids may play a role in the homodimerization of the protein. The product of a wild-type allele could be functionally hampered if dimerized with the product of a mutated allele. This is of particular significance when considering the discussion by Scott *et al.* (2000), which describes that the combination of certain SLC26A4 mutations could be dominant negative.

## Genetics of Pendred syndrome

Presently, more than 140 mutations in the *SLC26A4* gene have been described ([www.healthcare.uiowa.edu/labs/pendredandbor](http://www.healthcare.uiowa.edu/labs/pendredandbor)) in PS and EVA, which have been detected within the coding sequence, splice sites, non-coding exon 1, and within the *FOX11* binding transcriptional regulatory elements (Yang *et al.* 2007). Approximately 62% of all mutations are missense changes, most of which have been described in single families.





radioactive flux studies and localization in cells to assays utilizing intracellular fluorescent indicators to sense changes in halide and/or proton amounts (Table 2; it is important to note that the concentration of iodide in the different tests changes from very low (radioactive flux studies) to very high (fluorometric method)). The different techniques are consistent with each other and showed that entire proteins, not just the presumed transmembrane elements, are crucial for proper function. As shown in Fig. 5, deleterious mutations have been detected from amino acid 28 through amino acid 724 out of the 780 residue-long pendrin transporter. The hypothesis that the entire protein seems to be important is further substantiated in that all mutations leading to truncations are functionally inactive (Fig. 5). It is difficult at the moment to unambiguously link the severity of hearing loss (moderate, severe, and profound) to individual *SLC26A4* mutations. Mutations with no transport activity (P140H, Q413P, Q514K, and D724G) and with reduced activity (E29Q, V88I/R409H, G424D, and T485R) are equally identified in patients with PS and ns-EVA suffering from moderate to profound hearing loss (Taylor *et al.* 2002, Pera *et al.* 2008a). The simplest explanation for the difficulty in correlating a specific *SLC26A4* mutation to clinical symptoms is that additional genetic, epigenetic, and/or environmental factors could substantially modify the observed disease phenotype (Pera *et al.* 2008a). This is further corroborated in that *SLC26A4* zero mutants can show EVA and severe hearing loss, and patients with mono-allelic *SLC26A4* mutations can show severe to profound hearing loss without EVA (Pera *et al.* 2008a). Accordingly, Pryor *et al.* (2005) found that at least some cases of non-syndromic EVA are associated with a single *SLC26A4* mutation, whereas PS is a genetically homogeneous disorder caused by biallelic *SLC26A4* mutations. It should be noted that patients thought to have a monoallelic mutation may have an undetected mutation in a regulatory or intronic region (Gillam *et al.* 2005, Pryor *et al.* 2005). Despite the fact that it is difficult at the moment to correlate genotype (*SLC26A4*) and phenotype (moderate to profound hearing loss), it is noteworthy to mention that a patient homozygous for the Q514K mutation (loss-of-function) showed bilateral, sensorineural, and profound deafness at the age of 2, whereas a separate patient, we described, carrying two mutations with only reduced transport activity (E29Q and V88I/R409H), had sensorineural, moderate/mild hearing loss at the age of 44 (Pera *et al.* 2008a). These data indicate that the functional tests could indeed help explain the severity of particular pendrin mutations.

Our former work (Pera *et al.* 2008a) suggested that certain biochemical parameters could explain some functional impairments. For example, in all mutations

depicted in Fig. 5, an impairment in transport function was found if an amino acid bearing a fixed charge (aspartic acid (D<sup>-</sup>) or glutamic acid (E<sup>-</sup>), lysine (K<sup>+</sup>) or arginine (R<sup>+</sup>), and histidine (H<sup>+</sup>) single letter code, and the sign indicates the fixed charge; the positive charge of histidine depends on the pH) was missing or introduced. Furthermore, the loss or inclusion of a proline (P; proline acts as a structural disruptor of regular secondary structures such as  $\alpha$ -helices or  $\beta$ -sheets) in the *SLC26A4* sequence is detrimental for transport function. However, Pfarr *et al.* (2006) described one exception, in which an allelic variant (R776C; Fig. 5) functioned like the controls. Interestingly, this particular arginine is located on the extreme C-terminus of *SLC26A4* (a 780 amino acid protein). The N- and C-termini of a protein are usually not structurally defined; therefore, it is plausible to assume that mutations occurring in these areas probably have little or no functional impact.

It is important to note that mutations which do not enter into this 'proline/fixed charge' role can be functionally detrimental or without functional implication (Scott *et al.* 2000, Taylor *et al.* 2002, Gillam *et al.* 2004, Pfarr *et al.* 2006, Fugazzola *et al.* 2007). In Fig. 5, this applies for 12 out of the 36 mutations for which a functional reduction was described. In these roughly 33% of the cases, only functional tests can unambiguously distinguish between *SLC26A4* single-nucleotide-polymorphisms and those mutations that actually cause a reduced function and ultimately signs of disease.

In conclusion, it has become apparent that the two parameters used so far, i.e. i) low incidence of the mutation in the control population and ii) substitution of evolutionary conserved amino acids by the mutation, are not reliable for predicting *SLC26A4* transport function. The findings summarized here reveal that the 'proline/fixed charge' role, i.e. the addition or omission of proline, or the addition or omission of charged amino acids in the sequence of *SLC26A4*, might be a better option for predicting *SLC26A4* function in the cases where direct functional tests cannot be performed.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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