

The role of pendrin in iodide regulation

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Summary: Recent advances in human genetics have catalyzed the attention on Pendred's syndrome and its disease-gene, PDS. Studies on the expression of the PDS gene and on the function of its encoded protein, which has been named pendrin, are currently in progress. Consistent with the Pendred's syndrome phenotype, which is characterized by thyroid dysfunction associated to deafness, PDS expression has been demonstrated in the thyroid and in the inner ear. Despite its high homology to known sulfate transporters, pendrin has been shown to transport iodide and chloride, but not sulfate. Thus, it is probably devoted to regulate, at the apical membrane where it has been immunolocalized, the flux

of iodide from the thyroid cell to the colloid space. The function of pendrin in the inner ear is not well understood, but it seems to function also at this level as an anion transporter. Indeed, a pronounced PDS expression has been detected in structures of the inner ear, such as the membranous labyrinth and the endolymphatic duct and sac. At this level, the possible role of pendrin could be the maintenance of the appropriate ionic composition of the endolymph. – Although many questions remain to be answered, these recent achievements concerning the putative role of pendrin aid to better understand the genetic basis of the peculiar phenotype of Pendred's syndrome, which associate the dysfunction of two so different organs such as the thyroid and the inner ear.

The physiological role of pendrin

In 1997, a century after the first description of Pendred's syndrome, the disease gene has been mapped to chromosome 7q22-q31.1 and named PDS (Everett et al., 1997). It was identified using a positional cloning strategy and found to contain 21 exons and an ORF of 2,343 bp. The predicted protein, which has been named pendrin, consists of 780 amino acids (86 kD) and is highly hydrophobic (Everett et al., 1997). Computer modeling predicts that pendrin is a transmembrane glycoprotein containing 11 or 12 transmembrane domains. Pendrin belongs to a family of proteins, all of which appear to be anion transporters (Everett et al., 1997). In particular, there is a statistically significant homology with proteins known to function as sulfate transporters. Two of these proteins, DRA and DTD, which are implicated in congenital chloride diarrhea and diastrophic dysplasia (Silberg et al., 1995; Hastbacka et al., 1994) share a 45% and a 32% amino-acid identity with pendrin, respectively. Despite this significant sequence homology with the sulfate transport proteins, expression of pendrin in *Xenopus laevis* oocytes and Sf9 cells demonstrates that it does not transport sulfate but functions as a sodium-independent transporter of chloride and iodide (Kraiem et al., 1999; Scott et al., 1999).

The first Northern blot studies demonstrated that PDS is highly expressed in the thyroid and, to a lesser extent, in the fetal kidney and brain. PDS expression was also found in a human fetal cochlear cDNA library (Everett et al., 1997). Recent studies of immunohistochemistry (Bidart et al., 2000) have shown that pendrin is localized exclusively at the apical membrane of the thyroid cell. At this level, it has been hypothesized that pendrin could promote the transport of iodide from the cytoplasm to the colloid space, 'presenting' it to the TPO region involved in iodide organification (Fig. 1). In the normal tissue the expression of pendrin has been reported to be variable from follicle to follicle consistent with an heterogeneous follicular function (Royaux et al., 2000).

Pendrin has been reported to be highly expressed in Graves' disease and in toxic adenomas (Bidart et al., 2000; Royaux et al., 2000). Contrary to NIS and TPO mRNA expression, PDS mRNA expression is not augmented in these pathologies. PDS seems thus to be weakly sensitive to the TSH stimulating pathway. Furthermore, it has been reported that in FRTL-5 cells PDS expression is significantly induced by low concentrations of thyroglobulin, but not TSH, sodium iodide or insulin, suggesting a role of follicular thyroglobulin in the regulation of iodide metabolism (Royaux et al., 2000).

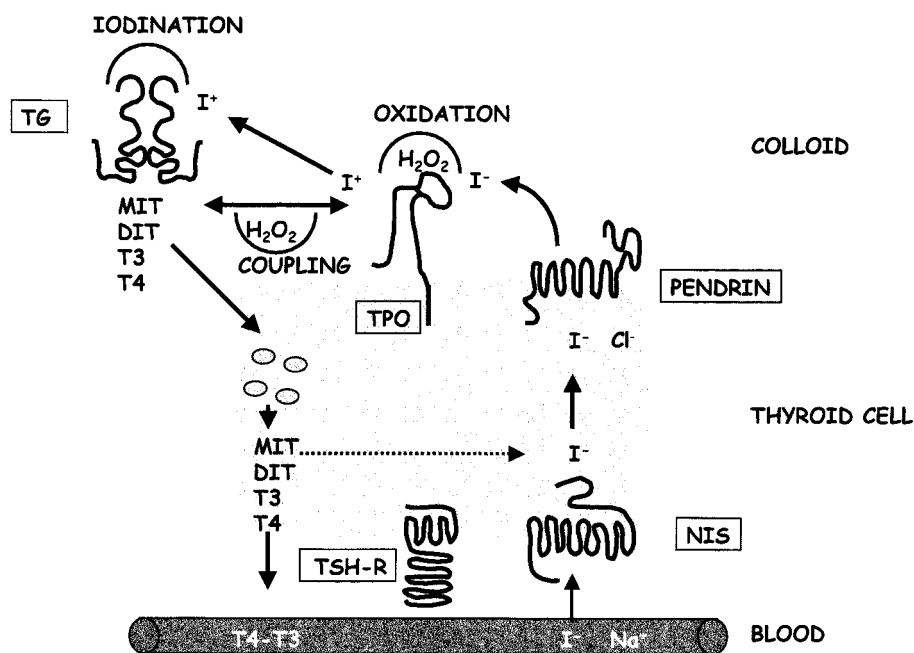


Fig. 1 Schematic representation of the thyroid cell with the iodine pathways. The TSH receptor (TSH-R) and the NIS protein are reported at the basal membrane. Pendrin and TPO are located at the apical membrane, while thyroglobulin (TG) occupies the colloid space

Recently, PDS expression has been also found in the inner ear of the mouse (Everett et al., 1999). In contrast to the emerging model for pendrin function in the thyroid, its role in the inner ear remains still obscure, even if it is likely that pendrin could transport anion(s) across the cellular membrane. Indeed, the most pronounced PDS expression has been found in the endolymphatic duct and sac (ED and ES). These structures contain endolymph which has a high potassium/chloride and very low sodium content (Ferrary and Sterkers, 1998). Endolymph is mainly secreted by the marginal cells of the stria vascularis, but its resorption is thought to occur in the ED and ES. Thus pendrin could function as an anion transporter maintaining the appropriate ionic balance within inner ear fluid, which is known to play a crucial role in the hearing process.

Finally, it has been reported that pendrin has transport properties similar to that of the renal chloride/formate exchanger, which participates in the resorption of filtered chloride in the proximal tubule of the kidney (Scott and Karniski, 2000).

The role of pendrin in Pendred's syndrome

In the last 3 years, more than 40 different PDS mutations have been reported in either homozygosity or compound heterozygosity in families with Pendred's syndrome (Everett et al., 1997; Coyle et al., 1998; Van Hauwe et al., 1998; Cremers et al., 1998; Coucke P et al., 1999; Kopp et al., 1999; Lopez-Bigas, 1999; Bogazzi et al., 2000; Fugazzola et al., 2000; Masmoudi, 2000). In the Figure 2, the position of each mutation along the putative pendrin structure is

shown. There is no clustering in any particular domain, though more than 40% of the mutations is localized in the C-terminus of pendrin (Fugazzola et al., 2000).

Pendred's syndrome was firstly described in 1896 as the association of goiter and deafness (Pendred, 1896). Further studies reported, in the following decades, that almost all Pendred's patients showed an abnormal perchlorate (KClO₄) discharge test, which is based on the measurement of the amount of radiolabeled iodide in the thyroid before and after the administration of KClO₄ (Morgans and Trotter, 1958). In a normally functioning thyroid gland inorganic iodide, having been trapped, is immediately organified by binding to thyroglobulin. The KClO₄ test unmasks defects of organification by provoking the discharge of inorganic iodide from the gland. In patients with Pendred's syndrome, the clearance of accumulated iodide is significantly higher (15–80%) as compared to normal controls (<10%) (Reardon et al., 1999). Unlike patients with defects in the Na⁺/I⁻ symporter (Fujiwara et al., 1998), patients with Pendred are able to accumulate iodide in the thyroid in a normal fashion, but binding to thyroglobulin is inefficient, consistent with the hypothesized role of pendrin at the apical membrane of the thyrocyte. Disruption of pendrin transport results in decreased iodide flux into the colloid and pooling of unbound iodide within thyrocyte cytoplasm.

Although occasional cases of false-positive results are known, the KClO₄ test is reliable (Reardon et al., 2000). On the contrary, goiter is not a constant feature being absent in almost 30–50% of reported cases (Reardon et al., 1997). An interfamilial and even intrafamilial variability can be observed also for the

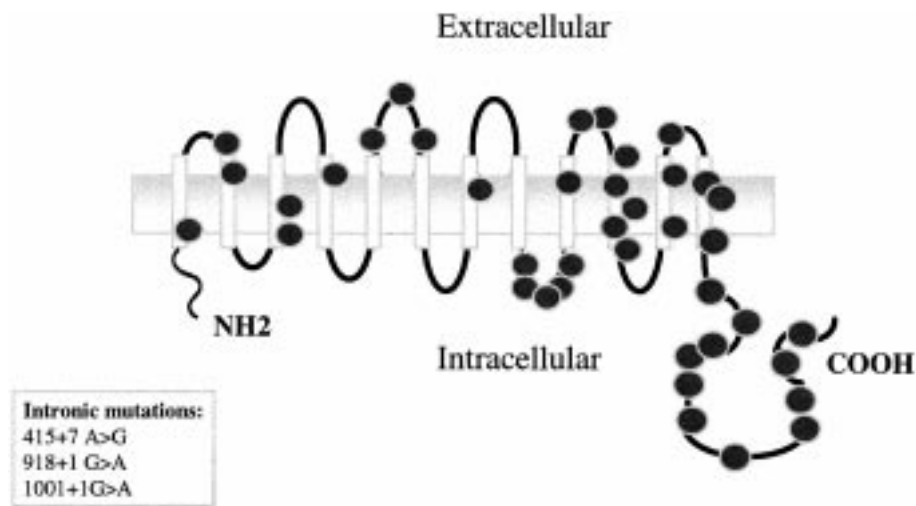


Fig. 2 Schematic representation of pendrin protein and the approximate position of all PDS mutations reported in the Literature. The intronic mutations are shown separately

thyroid function. Indeed, most patients are euthyroid, independently on the presence of goiter, while hypothyroidism, in general subclinical, is observed in about 30–40% of cases (Fraser, 1965; Reardon et al., 1999). It has been postulated that these phenotypic differences could be in relation to different degrees of iodine deficiency (Kopp et al., 1999).

An intriguing aspect of Pendred's syndrome is the fact that defects in a single gene and its encoded protein can lead to such divergent pathology as thyroid disease and deafness associated with a malformed inner ear. In Pendred's syndrome the deafness is sensorineural and occasionally accompanied by disturbed vestibular function (Reardon et al., 1999). It is associated with characteristic anomalies of the inner ear such as the Mondini cochlea (Mondini, 1791; Johnsen et al., 1986) and the enlargement of the vestibular aqueduct (Abe et al., 1999). More specific signs are the alterations of the membranous labyrinth and in particular the enlargement of the endolymphatic duct and sac (Johnsen et al., 1987; Phelps et al., 1998). Iodide is not of known importance in the inner ear, whereas a role for chloride can be readily postulated. In fact, a defect in the chloride transport properties of pendrin in the inner ear could contribute to the hearing loss associated with PS. A loss of chloride transport within the inner ear could lead to abnormal endolymph composition resulting in a damage of the neuroepithelium and in the enlargement of the membranous labyrinth structures with toxic and osmotic mechanisms (Qvortrup et al., 1999). Since ED and ES continue to mature until the age of 4, their enlargement could probably lead in some cases to an alteration of the surrounding bony structures, such as the vestibular aqueduct and the cochlea (Jackler and De La Cruz, 1989) (Fig. 3).

Further insights might possibly come by the understanding of pendrin role at the renal level. Chloride/formate exchange, in parallel with the Na^+/H^+ exchanger, provides a mechanism for NaCl and

volume resorption in the proximal tubule of the kidney (Schild et al., 1987), which could resemble the processes devoted to the maintenance of the electrolyte gradients and volume homeostasis within the inner ear. Renal abnormalities have not been described in Pendred's syndrome, but it is likely that clinically significant defects may be difficult to detect due to the redundancy of chloride transport processes along the nephron.

Conclusions

The recent advances in the genetic basis of Pendred's syndrome and the role of pendrin, are giving more insights into thyroid physiology and processing of hearing. At the thyroid level, the consequence of an impaired pendrin function is the deficient organification of iodide, probably due to the lack of iodide transport across the apical membrane, where the protein has been immunolocalized (Bidart et al., 2000). The accumulation of iodide into the thyroid cells leads to its discharge in response to perchlorate administration. If the putative role of pendrin at the apical membrane of the thyroid cell well explains the pathological KClO_4 test, more difficult to explain is the variability observed in thyroid function impairment and in the development of goiter. Even if a possible explanation could reside in different iodide intake, it is also possible that in the absence of pendrin function, a low level of iodide flux into the colloid space could still occur through a second transport system, such as an iodide channel (Dai et al., 1996; Golstein et al., 1992). In this way, sufficient iodide could be transported across the apical membrane to maintain thyroid function and prevent or postpone the onset of goiter. This second transport system that can compensate, at least in some individuals, for the decreased or absent pendrin function, is probably absent at the inner ear level and

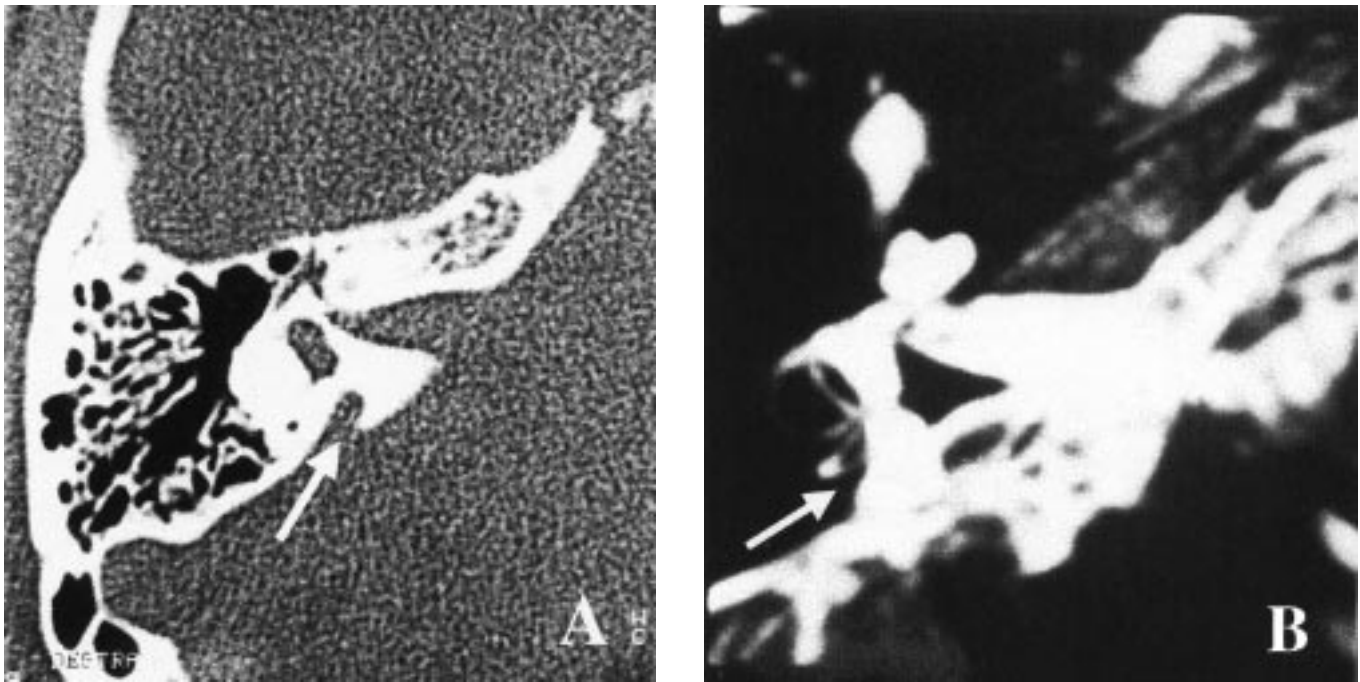


Fig. 3 High resolution CT scan and FSE-MRI of the petrous temporal bones in one patient with Pendred's syndrome. The enlargement of the endolymphatic sac (B) and of the corresponding vestibular aqueduct (A) are shown by arrows

the sensorineural hearing loss always develops. The differential sensitivity of these organs to the same mutation may be due, alternatively, to an amplified PDS expression in the thyroid with respect to the inner ear, allowing the thyroid gland to maintain a higher level of pendrin-mediated anion transport than the inner ear.

Indeed, individuals with disease-causing mutations in PDS can present with at least two distinct phenotypes. Most commonly, individuals have a 'classic' Pendred's syndrome phenotype of sensorineural hearing loss and goiter. Other individuals, however, show sensorineural hearing loss with inner ear malformations in the absence of goiter and with a normal KClO_4 test (Usami et al., 1999; Li et al., 1998). Recently, functional analysis had shown that mutations classically associated with Pendred's syndrome have complete loss of pendrin-induced chloride and iodide transport, while mutations associated to deafness without thyroid alterations are able to transport both iodide and chloride, even if at a lower level than the wild-type protein (Scott et al., 2000). It remains to be established the functional effect of those PDS mutations that are associated in some families to the complete Pendred's syndrome phenotype and in other families with isolated sensorineural hearing loss.

The desirable development of an experimental system such as a PDS-knockout mouse, should provide more insights on the role of pendrin in the thyroid, in the inner ear and in other tissues and

should aid to better define the phenotype of Pendred's syndrome.

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