Clinical and molecular characteristics of Pendred syndrome

Caractéristiques cliniques et moléculaires du syndrome de Pendred

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Résumé

Le syndrome de Pendred est une maladie autosomique récessive caractérisée par l'association de surdité neurosensorielle, par la présence d'un goitre et par un défaut partiel de l'organification de l'iode. Il est causé par des mutations bialéléiques du gène SLC26A4 qui code la protéine appelée pendrin. Au niveau de l'oreille interne, la pendrin joue un rôle important dans la formation de la composition de l'endolymphe et dans la génération du potentiel endocochléaire. Dans les cellules thyroïdiennes, la pendrin se trouve au pôle apical et elle joue un rôle dans l'éflux de l'iode dans la lumière folliculaire et/ou dans le contrôle du pH folliculaire. La présence du goitre et de la hypothyroïdie varient fortement et sont probablement influencées par la consommation de iodure. Au niveau du rein, la pendrin fonctionne comme échangeur de chlorure et de bicarbonate. La détection de la base moléculaire du syndrome de Pendred et de la fonction de pendrin a fourni des informations inattendues sur la pathophysiologie de l’oreille interne, la synthèse des hormones thyroïdiennes, ainsi que du contrôle rénal de l’échange de chlorure/bicarbonate.

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Mots clés : Syndrome de Pendred ; Pendrin ; SLC26A4 ; Surdité ; Goitre ; Hormones thyroïdiennes

Abstract

Pendred syndrome is an autosomal recessive disorder defined by sensorineural deafness, goiter and a partial defect in the organification of iodide. It is caused by biallelic mutations in the SLC26A4 gene, which encodes pendrin, a multifunctional anion exchanger. At the level of the inner ear, pendrin is important for the creation of a normal endolymph composition and the maintenance of the endocochlear potential. In the thyroid, pendrin is expressed at the apical membrane of thyroid follicular cells and it appears to be involved in mediating iodide efflux into the lumen and/or maintenance of the follicular pH. Goiter development and hypothyroidism vary among affected individuals and seem to be partially dependent on nutritional iodide intake. In the kidney, pendrin functions as a chloride/bicarbonate exchanger. Elucidation of the molecular basis of Pendred syndrome and the function of pendrin has provided unexpected novel insights into the pathophysiology of the inner ear, thyroid hormone synthesis, and chloride/bicarbonate exchange in the kidney.

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Keywords: Pendred syndrome; Pendrin; SLC26A4; Deafness; Goiter; Thyroid hormone

1. Introduction

Pendred syndrome (OMIM 274600; http://www.ncbi.nlm.nih.gov/omim/274600) is an autosomal recessive disorder defined by sensorineural deafness, goiter, and a partial defect in iodide organification. The syndromic association of goiter and deafness was first described by the British practitioner Vaughan Pendred in 1896 [1]. First insights into the pathophysiology underlying the thyroid phenotype were obtained by demonstrating that patients with Pendred syndrome have a partial iodide organification defect when submitted to a perchlorate discharge test [2]. In 1997, roughly one hundred years after the first description of the syndrome, the molecular cause was elucidated by demonstrating biallelic mutations in the Solute Carrier 26A4 (SLC26A4) gene, also referred to as Pendred Syndrome (PDS) gene, which encodes pendrin, a multifunctional anion transporter [3].
Pendred syndrome is thought to account for about 10% of hereditary hearing loss and is one of most common forms of syndromic deafness [4,5]. Estimated incidence rates are 7.5 to 10 in 100,000 individuals [4,5].

2. Clinical presentation

2.1. Inner ear phenotype

The sensorineural deafness is the hallmark of Pendred syndrome [2,6]. Most subjects present with profound prelingual hearing loss. In other individuals, however, the hearing impairment develops only later in life, often in a progressive manner [4,7]. Deafness is accompanied by malformations of the inner ear, which can be recognized readily by computed tomography or magnetic resonance imaging [8]. The endolympathic system is enlarged resulting in an enlarged vestibular aqueduct (EVA) [4,8,9]. Some patients present with a so-called Mondini cochlea [10], in which the coils of the cochlea are replaced by a single cavity [4,9]. The Mondini dysplasia is, however, not specific for Pendred syndrome and can be detected in several other disorders [11]. EVA is associated with syndromic and non-syndromic forms of sensorineural deafness [11]. The diagnosis of Pendred syndrome in patients with the finding of a uni- or bilateral EVA can be formally established by the demonstration of an iodide organification defect with the perchlorate test (see below), while goiter is a more variable sign. All patients with biallelic mutations in the SLC26A4 gene have Pendred syndrome, indicating that it is genetically homogeneous [11]. In contrast, patients with non-syndromic EVA are either homozygous for the SLC26A4 wild type gene, or they have only one mutated allele [11–13]. Interestingly, in some families, non-syndromic EVA is associated with monoallelic SLC26A4 mutations suggesting that unrecognized mutations in other regions of the gene, or in another gene, could contribute to the pathogenesis of the phenotype [11]. Consistent with the possibility of an oligogenic etiology in a subset of patients, double heterozygosity for mutations in the SLC26A4 gene and the transcription factor FOXI1, which is involved in the regulation of the SLC26A4 promoter, has been reported in a family with EVA, as well as in mice that are double heterozygous for disruption of these genes (Slc26a4+/−; Foxi1+/−) [14]. Digenic mutations in SLC26A4 and KCNJ10, a potassium channel involved in the generation of the endocochlear potential, in patients with EVA further support that this phenotype can have a oligo- or polygenic etiology [15].

2.2. Thyroid phenotype

Goiter development is highly variable among patients with biallelic mutations in the SLC26A4 gene and is sometimes not present at all [5,16]. However, all patients with biallelic mutations in the SLC26A4 gene have a partial iodide organification defect, irrespective of the presence or absence of a goiter, when submitted to a perchlorate discharge test [11]. The perchlorate test is used to determine whether iodide is normally organified [17]. Normally, less than 10% of radioiodide accumulated in thyrocytes are not organified into thyroglobulin [18]. In contrast, patients with Pendred syndrome loose more than 15% of accumulated radioiodine indicating that iodide organification is impaired [2,4]. It should be noted, however, that the organification defect is only partial and rather mild [2,11,19]. This differs from patients with a total organification defect, for example in the case of biallelic completely inactivating mutations in thyroid peroxidase (TPO) [18]. Despite the presence of a partial iodide organification defect, patients with Pendred syndrome may develop hypothyroidism under conditions of a low nutritional iodide intake, but are most commonly euthyroid if the iodide intake is high [5,20–23]. For example, patients with documented biallelic mutations in the SLC26A4 gene from countries with a high iodide intake such as Japan and Korea are always euthyroid [24–26]. In contrast, patients with Pendred syndrome from iodide-deficient regions may present with overt congenital hypothyroidism [27]. Many patients have elevated serum levels of thyroglobulin correlating with the size of the goiter [28,29].

2.3. Renal phenotype

Pendrin is abundantly expressed in the kidney, specifically at the luminal side of intercalated B cells of the cortical collecting duct and the connecting tubules [30,31]. Patients with Pendred syndrome and Slc26a4−/− mice have a normal renal function and do not display abnormalities in acid-base metabolism or fluid and electrolyte homeostasis under basal conditions suggesting that other anion exchangers can compensate for its absence [30,32,33]. As discussed below, pendrin appears to play an important physiological role in chloride retention and bicarbonate secretion [34].

3. The SLC26A4 gene and the pendrin protein

Pendrin belongs to the Solute Carrier Family 26A (SLC26A), which contains a group of multifunctional anion channels [35]. With the exception of the motor protein of the outer hair cells, prestin, all members of this family function as anion exchangers [35–37]. The two human genes most closely related to pendrin are SLC26A3 (also referred to as down-regulated in adenoma [DRA]) and SLC26A2 (also referred as diastrophic dystrophy [DTD]) [38,39]. SLC26A3/DRA encodes a chloride/sulfate transporter, which is abundantly expressed in the intestine and the prostate [39,40]. The gene encoding SLC26A3/DRA is located in a head-to-head orientation in close proximity to the SLC26A4 gene, suggesting a common ancestral gene [41]. Mutations in the SLC26A3/DRA gene cause congenital chloride diarrhea (CLD) [39]. SLC26A2/DTD is a sulfate transporter that is predominantly expressed in the intestine and cartilage; mutations in this gene cause several chondrodysplasias [42]. The SLC26A4 gene consists of 21 exons and is located on chromosome 7 [3]. Pendrin is a membrane protein consisting of 780 amino acids that is glycosylated [43–45]. It is predicted to have 12 putative transmembrane domains with both the amino- and carboxy-termini facing the cytosol [19,44]. Pendrin is known to have a sulfate transporter domain and a sulfate transporter and antisigma factor antagonist (STAS) domain [46]. The STAS domain has been suggested to play a role in nucleotide
binding or to interact with other proteins such the cystic fibrosis conductance regulator (CFTR), but its exact role remains to be elucidated [46–49].

4. Function of pendrin

Pendrin is able to mediate exchange of chloride with bicarbonate, formate, and iodide [31,43,50,51]. Pendrin is mostly abundantly expressed in the thyroid, the inner ear and the kidney where it seems to have specific physiological roles [31,44,52].

4.1. Function in the inner ear

Several studies explored the expression and function of pendrin in the inner ear in the mouse [52–56]. In the developing inner ear, Slc26a4 mRNA is expressed in specific areas of the endolympathic duct and sac, the utricle and saccule [52]. These regions are important for maintaining the composition and resorption of endolymph, which is a prerequisite for normal function of the inner ear.

Slc26A4 deficient mice are completely deaf and display a vestibular phenotype with tilting of the head, an unsteady gait, circling, and an abnormal reaching response [56]. Anatomically, the inner ear of these animals develops normally until embryonic day 15, but then dilatation of the endolympathic duct and sac leads to degeneration of sensory cells, and malformation of otoconia and otoconial membranes [56]. The Slc26a4−/− mice lack the endocochlear potential as a result of a loss of expression of the potassium channel Kcnj10 located at the membrane of the intermediate cells [54,55]. The loss of pendrin-mediated exchange of chloride with bicarbonate also leads to acidification of the endolymph, which in turn results in the inactivation of apical calcium channels (TRPV5 and TRPV6) [53]. Inactivation of these channels regulating calcium absorption in the vestibular system results in an increased calcium concentration in the endolymph [53].

Mice with targeted disruption of the winged helix/forkhead gene Foxi1 lack pendrin expression in the inner ear and develop a phenotype that resembles the Slc26a4−/− mouse model [57]. These findings indicate that Foxi1 is a transcriptional regulator of the SLC26A4 gene [57], a finding that is in line with the observation that humans with digenic mutations in the SLC26A4 and the FOXII genes develop a Pendred syndrome phenotype [14].

4.2. Function in the thyroid

The synthesis of thyroid hormones is dependent on thyrocytes forming an intact follicle [18]. At the basolateral membrane of thyrocytes, the sodium-iodide symporter (NIS) mediates iodide uptake [58] in conjunction with the sodium gradient generated by the Na+K+-ATPase [59]. It then reaches the follicular lumen at the apical membrane, presumably at least in part facilitated by pendrin [19]. Electrophysiological studies characterizing iodide efflux in inverted plasma membrane vesicles suggested, however, the existence of two apical iodide channels [60].

Subsequently, iodide is oxidized and organized by incorporation into selected tyrosyl residues of thyroglobulin. This reaction, referred to as organification, is catalyzed by TPO in the presence of hydrogen peroxide (H2O2), and results in the formation of mono- and diiodotyrosines (MIT, DIT). In the subsequent coupling reaction, which is also catalyzed by TPO, two iodotyrosines are fused by an ether bond to form either thyroxine (T4) or triiodothyronine (T3). Iodinated thyroglobulin is then entering the cells by pinocytosis and digested in lysosomes. Released T4 and T3 are secreted into the bloodstream at the basolateral membrane, in part by MCT8 [61]. MIT and DIT are subjected to deiodination by an intracellular iodotyrosine dehalogenase (DEHAL1) and the released iodide is recycled back into the follicular lumen [18,62,63].

In the thyroid, pendrin is expressed at the apical membrane of thyroid follicular cells [44]. The expression of pendrin at the apical membrane of thyrocytes and its ability to transport iodide suggested that pendrin could represent one of these iodide channels mediating apical iodide efflux. Moreover, the partial iodide organification defect observed in patients with biallelic SLC26A4 mutations is consistent with a potential role of pendrin in thyroid hormone synthesis.

Initial functional studies of pendrin in Xenopus oocytes have shown that pendrin is unable to transport sulfate despite its homology to sulfate transporters but that it can mediate uptake of chloride and iodide in a sodium-independent manner [51]. The ability of pendrin to mediate iodide efflux is now supported by a number of studies using heterologous expression systems with non-polarized cells [19,64–66], as well as polarized cells [19]. For example, iodide efflux is much higher in non-polarized Chinese hamster ovary (CHO) cells coexpressing NIS and pendrin compared to cells expressing NIS alone [65]. Electrophysiological studies of COS-7 cells transfected with pendrin indicate that iodide efflux is more efficient under high extracellular iodide concentrations suggesting the possibility of an exchange of iodide with chloride [66]. Moreover, findings obtained in polarized Madin-Darby canine kidney (MDCK) cells support the concept that pendrin plays a role in facilitating vectorial iodide transport at the apical membrane into the follicular lumen [19].

There are, however, remaining controversies regarding the role of pendrin in mediating apical iodide transport. Targeted disruption of pendrin in mice does not lead to the development of a goiter or abnormal thyroid hormone levels [56], even under conditions of iodine deficiency [67]. Moreover, it remains intriguing that pendrin seems to have distinct roles in the thyroid, compared to its role as a chloride/bicarbonate exchanger in the kidney [30,31], and the inner ear [50,52]. Next, it should be noted that patients with biallelic mutations in the SLC26A4 gene have only a mild or no thyroidal phenotype under normal iodide intake conditions [23]. Lastly, there are no functional data demonstrating a role of pendrin in iodide transport in vivo. For all these reasons, it is conceivable that other iodide channels and/or transporters are involved in the apical transport of iodide. Other proteins have been proposed to mediate apical iodide efflux. Specifically, they include SLC5A8 and the chloride channel 5, CLCN5 [68,69]. However, SLC5A8 (originally
designated as human apical iodide transporter (hAIT) [69]) is not involved in mediating iodide efflux as demonstrated by functional studies in *Xenopus* oocytes and polarized MDCK cells [68]. Localization of the CICn5 protein at the apical membrane of thyrocytes and the development of a thyroidal phenotype in CICn5-deficient mice similar to Pendred syndrome, suggests that CICn5 could participate in mediating apical iodide efflux or iodide/chloride exchange, perhaps in conjunction with other chloride channels [70]. Further functional studies are, however, needed to characterize the potential role of CICn5.

Several studies have demonstrated that the efflux of iodide across the apical membrane of thyrocytes is stimulated by TSH through activation of the cAMP pathway [71–73]. In polarized porcine thyrocytes cultured in bicameral chambers, TSH upregulates iodide efflux selectively at the apical membrane of the cells [71]. Following short-term exposure to insulin, translocation of pendrin occurs from the cytosol to the plasma membrane via a protein kinase C-ε (PKC-ε) dependent pathway in rat thyroid PCC13 cells [74]. Another study demonstrated that exposure to TSH rapidly upregulates pendrin protein insertion at the plasma membrane and leads to an increase in iodide efflux [75]. This effect occurs within minutes and is mediated through the protein kinase A (PKA) pathway [75].

Although TSH regulates apical iodide efflux, it does not stimulate the expression of SLC26A4 mRNA [44]. Similarly, insulin does not stimulate SLC26A4 expression [44]. However, TSH and insulin appear to enhance SLC26A4 gene expression in the presence of thyroglobulin [76]. In contrast, TG suppresses expression of several essential thyroid-restricted genes including *TSHR*, *NIS*, *TPO*, *TG*, *PAx8*, *TF1*, and *TF2* [77]. It has been proposed that thyroglobulin, by mediating differential expression of these genes, regulates the rate of iodide efflux into the follicular lumen, thereby regulating thyroid hormone synthesis under constant levels of TSH [76].

4.3. Function in the kidney

In the kidney, pendrin resides on the apical membrane of intercalated type B cells, and in intercalated type non-A-non-B cells in the cortical collecting duct and the connecting tubules [30,31]. Type B cells are known to secrete bicarbonate, while type A cells mediate hydrogen secretion [34]. The physiological role of type non-A-non-B cells remain to be defined [34].

Pendrin has been shown to mediate bicarbonate secretion [30], and it contributes to the regulation of blood pressure by modulating renal chloride absorption [32,78]. In mice, pendrin expression is significantly increased during metabolic alkalosis and under these conditions it is predominantly localized at the apical membrane [79]. In contrast, under conditions of metabolic acidosis, pendrin expression decreases and results in the translocation of pendrin from the apical membrane into cytosolic compartments. Renal tubules isolated from alkali-loaded wild type mice show normal secretion of bicarbonate, whereas tubules from alkali-loaded pendrin knockout mice are unable to secrete bicarbonate [30]. Taken together, these results suggest that pendrin is involved in bicarbonate secretion during metabolic alkalosis. As mentioned, patients with Pendred syndrome and pendrin knockout mice have a normal renal function and do not display abnormalities in acid-base metabolism or fluid and electrolyte homeostasis under basal conditions. This indicates that other chloride-base exchangers can compensate for the loss of pendrin [30,32,33].

Pendrin has also been implicated in renal chloride absorption in response to certain stimuli. The level of pendrin expression is upregulated in response to treatment with the aldosterone analogue deoxycorticosterone pivalate (DOCP) and following dietary chloride restriction [32,33]. Aldosterone increases blood pressure and vascular volume by stimulating renal sodium and chloride absorption. During sodium chloride restriction, which increases aldosterone levels, *Slc26a4−/−* mice have a lower blood pressure and a more severe metabolic alkalosis compared to wild type mice [80]. This is thought to be secondary to the decreased ability of the knockout mice to absorb chloride and secrete bicarbonate [80]. During salt restriction, the function and protein abundance of ENaC is significantly reduced in *Slc26a4−/−* mice [81].

Taken together, these findings suggest that pendrin is involved in the regulation of electrolyte homeostasis and blood pressure by mediating net acid and chloride excretion [32,80]. For these reasons, pendrin may be a potential target for the treatment of hypertension [32,80].

5. Mutations of pendrin

Numerous mutations in the *SLC26A4* gene, dispersed throughout the gene, have been reported in patients with Pendred syndrome (http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm).

The majority are missense mutations, a much smaller portion are nonsense, splice site, and frameshift mutations [82]. Patients from inbred families are homozygous for a given mutation in the *SLC26A4* gene. In non-consanguineous families or sporadic cases, it is more common to find compound heterozygous mutations [83]. The frequency of recurring mutations varies among different ethnic groups [25]. L236P, T416P, IVS8 + 1G > A occur relatively frequently in European populations [84], H723R is very common in patients from Korea and Japan [24–26].

It has been shown that the loss of function of some of these mutations results from the retention of the mutated and misfolded proteins in the endoplasmic reticulum [45]. It has been suggested that the processing of pendrin mutant proteins involves different intracellular mechanisms that are specific for each mutant protein, in part because of a differential degree of N-glycosylation [85]. Some mutations can be rescued by treatment with the aldosterone analogue deoxycorticosterone pivalate (DOCP) and following dietary chloride restriction [32,33].

6. Pendrin in other tissues

Expression of pendrin has been documented predominantly in the thyroid, the inner ear, and the kidney, but also in few other tissues.
In the placenta, pendrin protein expression has been documented at the brush border of syncytiotrophoblast cells facing the maternal side, while NIS is expressed on the entire membrane of the cytrophoblast [87]. The role of NIS and pendrin in the placenta have not been further characterized.

In the lactating breast, both NIS and pendrin are expressed [88]. NIS is located at the basolateral membrane during lactation, indicating that it is mediating the uptake of iodide, which is secreted into the milk [58]. The subcellular location and physiological importance of pendrin in the alveolar cells of the lactating mammary gland remain to be defined.

Very low levels of SLC26A4 mRNA expression have been reported in tissues such as the lung, prostate, endometrium, and Sertoli cells [89,90]. In the lung, pendrin expression increases after treatment with IL-4 and it has been proposed to be involved in the transport of thiocyanate [90].

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


