Pendred Syndrome in Two Galician Families: Insights into Clinical Phenotypes through Cellular, Genetic, and Molecular Studies

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Context: We studied two families from Galicia (northwest Spain) with Pendred syndrome (PS) and unusual thyroid phenotypes. In family A, the proposita had a large goiter and hypothyroxinemia but normal TSH and free T₃ (FT₃). In family B, some affected members showed deafness but not goiter.

Objective: Our objective was to identify the mutations causing PS and molecular mechanisms underlying the thyroid phenotypes.

Interventions: Interventions included extraction of DNA and of thyroid tissue.

Patients: Propositi and 10 members of the two families participated in the study.

Main Outcome Measures: Main outcome measures included SLC26A4 gene analysis, deiodinase activities in thyroid tissue, and c.416–1G → A effects on SLC26A4 splicing. In addition, a primary PS thyrocyte culture, T-PS2, was obtained from propositus B and compared with another culture of normal human thyrocytes, NT, by Western blotting, confocal microscopy, and iodine uptake kinetics.

Results: Proposita A was heterozygous for c.578C → T and c.279delT, presented with goiter, and had normal TSH and free T₃ (FT₃). Proposita B bore c.279delT and a novel mutation c.416–1G → A; some deaf relatives were homozygous for c.416–1G → A but did not present goiter. The c.279delT mutation was associated with identical haplotype in the two families. T-PS2 showed truncated pendrin retained intracellularly and high iodine uptake with low efflux leading to iodine retention.

Conclusions: c.279delT is a founder mutation in Galicia. Proposita A adapted to poor organification by increasing deiodinase activities in the goiter, avoiding hypothyroidism. Lack of goiter in subjects homozygous for c.416–1G → A was due to incomplete penetrance allowing synthesis of some wild-type pendrin. Intracellular iodine retention, as seen in T-PS2, could play a role in thyroid alterations in PS. (J Clin Endocrinol Metab 93: 267–277, 2008)

Pendred syndrome (PS) is an autosomal recessive disorder characterized by congenital sensorineural hearing loss and goiter without or with hypothyroidism (1). SLC26A4 (solute carrier family 26, member 4), the PS gene (2), encodes a transmembrane protein (pendrin) expressed in the thyroid gland, inner ear, endometrium, and kidney, where it is involved in iodide,...
chloride, formate, and nitrate transport (3). *SLC26A4* mutations are also implicated in neurosensory nonsyndromic recessive deafness 4, DFNB4, with inner ear malformations (4, 5). Around 150 mutations in *SLC26A4* have been reported (http://www.medicine.uiowa.edu/pendredandbor/listed_mutations.htm). Different populations are affected by different mutations, and founder mutations have been reported in a few cases (4–17).

In the thyroid gland, pendrin acts at the apical pole of thyrocytes to transport intracellular iodide into the follicular lumen (18). Loss of pendrin function causes a failure in iodine supply and an organification defect often leading to euthyroid goiters (8, 10, 12, 16, 19) similar to those seen in iodine-deficient areas (20). We report two unrelated families with PS who have a thymidine deletion c.279delT at exon 3, resulting from a founder mutation. A thyrocyte cell line, T-PS2, was obtained from a primary thyroid culture of the family B propositus, providing data on the effects of the c.279delT and c.416-1G→A mutations on mutated pendrin localization and iodine handling in affected thyrocytes.

### Subjects and Methods

#### Subjects

**Family A**

The proposita was a 43-yr-old deaf woman with grade III asymmetric multinodular goiter (Fig. 1). Neither her parents nor her three siblings were deaf. Although serum free T4 (FT4) was low (0.51 ng/dl, 6.56 pmol/liter; normal range 0.85–1.69 ng/dl), her serum TSH, FT3, and rT3 were normal; serum thyroglobulin (Tg) was 1312 ng/ml (normal range 0–80 ng/ml), anti-thyroglobulin (anti-TPO) and anti-Tg antibodies were negative, and urine iodine was 102 μg/liter (median value for her age in our population is 79.7 μg/liter). A computer tomography scan showed enlarged vestibular aqueducts. A perchlorate test showed an organification defect. Increasing daily doses of L-thyroxine (25, 50, 75, and 100 μg) were given, but her serum FT4 levels remained low or low-normal. A total thyroidectomy was performed (Fig. 1), and the patient was discharged on 100 μg L-thyroxine daily.

**Family B**

The propositus, a 26-yr-old deaf male, was referred to us for hypothyroidism. He was the only child of a nonconsanguineous deaf couple...
with a strong family history of deafness and goiter (Fig. 2). He had a goiter with a 1-cm nodule in the right lobe. Serum TSH was 7.08 μU/ml, with low FT4 (0.73 ng/dl, 9.40 pmol/liter) and normal FT3. Anti-TPO and -Tg antibodies were negative. A fine-needle aspiration cytology was suggestive of follicular neoplasia, and a right hemithyroidectomy was performed. The propositus’ mother had a grade II goiter, whereas his father had neither goiter nor abnormal serum levels of thyroid hormone, although a perchlorate test did show a partial organification defect. Both parents presented with profound neonatal deafness, as did two of the mother’s four siblings and two of the father’s seven siblings. One of the father’s brothers (subject IIIB.3, Fig. 2) showed much less severe deafness starting in childhood.

Genetic studies

Genomic DNA was extracted from blood cells of the propositi, 10 members of their families, and 50 normal volunteers (age range 20–60 yr) and from thyroid tissues of the propositi and 60 control subjects (healthy parts of surgically removed multinodular goiters). All exons of the SLC26A4 gene were amplified by PCR (primer sequences and PCR conditions available upon request) and sequenced in an ABI PRISM 3100 (Applied Biosystems, Foster City, CA). The study was approved by our Institutional Review Board, and informed consents were obtained.

For haplotype analysis, four polymorphic markers closely linked to SLC26A4 were genotyped. According to the NCBI STS map, D7S2459 is located in SLC26A4 intron 10, and D7S2420 and D7S496 are proximal and D7S2456 distal to SLC26A4. Oligonucleotide primer sequences were obtained from http://www.ncbi.nlm.nih.gov, and forward primers were fluorescence labeled. PCR products were electrophoresed in a MegaBace 500 (Amersham Pharmacia Biotech, Piscataway, NJ). Alleles were numbered according to product size.

Effects of the intronic mutation on SLC26A4 were investigated in skin fibroblasts and thyroid tissue from the B propositus. cDNA fragments spanning from exon 3 to exon 6 were PCR amplified and cloned into a pGEMT-Easy vector (Promega, Madison, WI), and the products were sequenced as described.

D1 and D2 activities were measured in thyroid tissue homogenates as described (22, 23).

Thyroid hormone levels in thyroid gland samples were determined by in-house RIAs (24).
histological and immunohistochemical studies

Immunohistochemical studies were performed on paraffin sections of thyroid specimens from the two probands using an EnVision peroxidase/diaminobezide kit with antibodies to thyroid transcription factor-1 (Dako, Carpinteria, CA; dilution 1:50), Tg (Tg6, 1:2000; Dako), TPO (MoAb47, 1:50; Dako), calcitonin (polyclonal, 1:1000; BioGenex, San Ramon, CA), cytokeratin (CK) 7 (OV-TL 12/30, 1:50; Dako), CK1–5 (MoAb47, 1:50; Dako), calcitonin (polyclonal, 1:1000; BioGenex), and calretinin (1% for 10 min at room temperature, and then quenched with 50 mM NH4Cl for 1 h. Alternatively, cells were fixed with ice-cold methanol for 10 min. Antibodies used were the Chemicon anti-NIS (1:50) and PS1Ab (1:20), methanol-fixed cells) or PS5Ab, which recognizes the last 13 carboxyl-terminal amino acids of human pendrin, denominated pendrin5 (1:20, paraformaldehyde-fixed cells) (25). Thyrocytes were identified by Tg immunofluorescence (Novocastra; 1:65). The nucleus was counterstained with 4',6-diamidino-2-phenylindole (DAPI) (Sigma; 1:100).

iodide uptake

Iodide uptake was measured according to Dohan et al. (29) with minor modifications, using T-P2 and NT cells grown in 24-well plates. For steady-state experiments, incubations proceeded for 30 min with 20 or 40 μM Na125I. For time-course analysis, cells were incubated for 30 sec and 1, 2, 5, 10, 15, and 30 min. For dose-response experiments, cells were incubated for 30 sec and 5, 30, and 60 min with 0.1, 0.25, 0.5, 1.25, 2.5, 5, 10, 20, or 40 μM NaI. Cells were lysed by adding 200 μl 1 M NaOH to each well for 10 min at room temperature. 125I in cells was then quantitated in a γ-scintillation counter. Cells from replicate wells were counted to express 125I uptake as pmol/cm2 cells. NaClO4 (40 μM) was added to inhibit I− uptake when appropriate.

For efflux experiments, cells were loaded with 20 μM Na125I for 30 min and washed (29); some replicates were terminated at this point (intracellular 125I content 100%), whereas in the other replicates, the medium was replaced at 5, 15, and 25 min as described (29). Radioactive medium was quantitated, and results are expressed as percentage of intracellular content. Finally, cells were lysed for quantitation of 125I.

Kinetic curves were fitted by nonlinear least-square regression using GraphPad Prism software based on the Michaelis-Menten equation. All parameters were determined at least in triplicate in three independent experiments.

<table>
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<th>Expected protein</th>
<th>Mutation</th>
<th>Size (bp)</th>
<th>mRNA transcript</th>
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<tr>
<td>Premature stop</td>
<td>r.279delT</td>
<td>499</td>
<td>r.279delT</td>
</tr>
<tr>
<td>Wild-type pendrin</td>
<td></td>
<td></td>
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<td>500</td>
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<td>Premature stop</td>
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<tr>
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<td>420</td>
<td></td>
</tr>
<tr>
<td>Premature stop</td>
<td>5</td>
<td>417</td>
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</tbody>
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slips, fixed with 1% paraformaldehyde for 20 min, permeabilized with Triton X-100 for 10 min at room temperature, and then quenched with 50 mM NH4Cl for 1 h. Alternatively, cells were fixed with ice-cold methanol for 10 min. Antibodies used were the Chemicon anti-NIS (1:50) and PS1Ab (1:20), methanol-fixed cells) or PS5Ab, which recognizes the last 13 carboxyl-terminal amino acids of human pendrin, denominated pendrin5 (1:20, paraformaldehyde-fixed cells) (25). Thyrocytes were identified by Tg immunofluorescence (Novocastra; 1:65). The nucleus was counterstained with 4',6-diamidino-2-phenylindole (DAPI) (Sigma; 1:100).

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Data are shown as means ± sd. Numbers 1–7 refer to healthy parts of surgically derived thyroid specimens. ND, Not done; T, thyroid tissue from inside a toxic adenoma.

Sample | MCT8 | D1 | D2 | D1 (pmol/min/mg protein) | D2 (fmol/h/mg protein)
<table>
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<tr>
<td>Family A proposita</td>
<td>1.00 ± 0.05</td>
<td>1.00 ± 0.07</td>
<td>1.00 ± 0.08</td>
<td>70.7 ± 0.1</td>
<td>166.5 ± 34.9</td>
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<tr>
<td>1</td>
<td>0.93 ± 0.09</td>
<td>0.37 ± 0.05</td>
<td>0.54 ± 0.10</td>
<td>11.2 ± 1.6</td>
<td>86.3 ± 18.8</td>
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<tr>
<td>2</td>
<td>0.36 ± 0.03</td>
<td>0.11 ± 0.01</td>
<td>0.16 ± 0.12</td>
<td>6.8 ± 0.3</td>
<td>32.5 ± 5.3</td>
</tr>
<tr>
<td>3</td>
<td>0.45 ± 0.02</td>
<td>0.24 ± 0.01</td>
<td>0.21 ± 0.03</td>
<td>45.4 ± 3.8</td>
<td>132.9 ± 12</td>
</tr>
<tr>
<td>4T</td>
<td>0.90 ± 0.20</td>
<td>0.64 ± 0.11</td>
<td>0.29 ± 0.06</td>
<td>142 ± 4.3</td>
<td>180 ± 2</td>
</tr>
<tr>
<td>5</td>
<td>0.30 ± 0.03</td>
<td>0.26 ± 0.02</td>
<td>0.55 ± 0.10</td>
<td>24.7 ± 4.3</td>
<td>54.8 ± 22.7</td>
</tr>
<tr>
<td>6</td>
<td>1.63 ± 0.04</td>
<td>0.78 ± 0.07</td>
<td>0.59 ± 0.17</td>
<td>34.2 ± 4.1</td>
<td>150.7 ± 0.6</td>
</tr>
<tr>
<td>7</td>
<td>0.91 ± 0.01</td>
<td>0.55 ± 0.22</td>
<td>0.18 ± 0.14</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
Thyroid function tests

TSH, FT₄, and FT₃ were measured by chemiluminescence using ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY). Tg, TgAb, and TPOAb were measured using Immulite 2000 (Diagnostic Products Corp., Los Angeles, CA). rT₃ was measured by RIA (Biocode Hycel, Liege, Belgium).

Statistical analysis

One-way ANOVA with post hoc comparisons by Student’s t test and the Wilcoxon signed-rank test were used for statistical analysis.

Results

Genetic studies

The family-A proposita was heterozygous for c.279delT and c.578C→T. Mutation c.279delT (Fig. 1D), located in exon 5, results in replacement of the normal thymine with an isoleucine at codon 193 (p.Thr193Ile) in the third membrane region of pendrin. The proposita inherited mutation c.578C→T from her mother. No mutations were found in the proposita’s sister, and no DNA samples were available from her father or two brothers.

The family B propositus was heterozygous for c.279delT (see above) and for c.416–1G→A, located at the acceptor splice site of intron 4, leading to replacement of the normal guanosine with an adenosine. His mother had the same compound heterozygous genotype (c.279delT, c.416–1G→A) and his father was homozygous for c.416–1G→A (Fig. 2). Two of the father’s siblings (IIIB.1 and IIIB.2, Fig. 2) showed profound deafness and were homozygous for c.416–1G→A. Subject IIIB.3 (Fig. 2) did not have SLC26A4 mutations and showed much less severe deafness.

Mutations c.279delT, c.578C→T, and c.416–1G→A were not found in 120 alleles from 60 normal thyroid tissue samples obtained from Galician patients or in 100 alleles from 50 blood samples obtained from normal Galician volunteers aged between 20 and 60 yr.

Members of the two families bearing c.279delT shared the same haplotype, which was not present in unaffected individuals (Fig. 2). Regarding c.416–1G→A, both affected and unaffected members of family B share a common haplotype not found in family A (Fig. 2).

PCR amplification of propositus B thyroid SLC26A4 cDNA, extending from exon 3 to exon 6, gave the expected 500-bp product and a 420-bp product. A similar result was obtained using fibroblast cDNA from this propositus. In control thyroid tissue, only a 500-bp product was observed. The 500-bp fragment corresponded to three transcripts (Table 1): a 499-bp transcript [r.279delt (37.5%)] resulting from thymidine deletion at nucleotide 279, a 499-bp transcript [r.416–1 g→a;416_417del (25%)] resulting from an abnormal splicing one base from the regular splicing site, and an unexpected 500-bp wild-type transcript (37.5%). The 420-bp fragment likewise corresponded to two transcripts, of 420 and 417 bp, that resulted from abnormal splicing 79 and 82 bp from the regular site (r.416–1 g→a; 416_495del, 416_498del). All the abnormal transcripts introduced premature stop codons.

Deiodinase and MCT8 levels

Deiodinase mRNA expression and activities were higher in the thyroid gland of the A proposita than in most control thyroid tissues (Table 2). MCT8 mRNA expression was also high in the proposita’s thyroid (Table 2). Thyroid hormone contents in con-
FIG. 4. At the outer plasma membrane, normal thyrocytes (NT) express fully glycosylated NIS and pendrin, whereas cells from the propositus of family B (T-PS2) express only NIS. A, The appearance of cultured NT (a) and T-PS2 cells (b) is similar under the phase-contrast microscope. The cells are small and polygonal and leave round spaces between them, recalling a follicular structure (see arrows). In both cell lines, using DAPI for nuclear counterstaining, practically all cells expressed Tg (shown for T-PS2, c and d). B, Western blotting against NIS (left) and pendrin (right) using hot SDS extracts enriched in plasma membrane proteins (membrane) or 1% Triton extracts with intracellular membrane content, i.e., Golgi or ER (cytoplasmic). As a loading control, the membranes were rehybridized against tubulin. NIS is expressed mainly as the 100-kDa fully glycosylated form at the plasma membrane in NT, T-PS2, and the T-FA6 primary-culture follicular adenoma line from our BANTTIC collection; smaller bands around 80 kDa correspond to nonglycosylated immature NIS. Intracellular levels are undetectable in all lines except NT, in which a faint band can be seen suggesting slightly greater NIS expression. Mouse 3T3 fibroblasts were used as negative control. NT cells express the 130-kDa fully glycosylated pendrin at the plasma membrane, but only a very weak band (less than 5%) can be seen in T-PS2 and T-FA6 cells. The faint 85-kDa band is the nonglycosylated protein.
obtained through stacking of confocal images show that, although localized in the membrane, the two proteins rarely coincide in the same locations, as indicated by the yellow spots and arrows in Figure 4A. Confocal immunofluorescence studies with PS1Ab showed staining of NT thyrocytes at a point near the nucleus in the Golgi location and in narrow lines typical of plasma membrane localization (Fig. 4C, e and f). Almost all T-PS2 thyrocytes showed the spot near the nucleus (Fig. 4G, g and h), but no lines were detected, indicating either that normal and truncated proteins were both retained in the Golgi or that the concentration of normal pendrin is very low at the membrane. Recent results in our laboratory have shown low levels of pendrin mRNA expression in T-PS2 compared with NT (unpublished results), suggesting that the weak membrane expression of pendrin in T-PS2 could be related not only to defective membrane targeting but also to low transcription levels of the pendrin mutants.

We also studied the colocalization of NIS and pendrin (using PS1Ab against pendrin1 and PS5Ab against pendrin5). In normal thyrocytes, both NIS and pendrin1 showed a linear staining typical of plasma membrane localization (Fig. 4D, i–l). However, the two proteins were usually not expressed in the same membrane patches, as can be seen from the scarce colocalization in the projections and the z planes. In T-PS2 thyrocytes, NIS was also located in the plasma membrane, but pendrin1 showed very few spots outside the Golgi (Fig. 4D, m–p). Next, we repeated the colocalization studies using PS5Ab, recognizing the last 13 carboxyl-terminal amino acids of human pendrin. In these studies, both NIS and pendrin showed linear staining in the NT thyrocytes, but again, each protein localized in its plasma membrane region, with little colocalization (Fig. 4D, q–t). Although the cells were grown in monolayers, this arrangement recalls that of partially polarized thyrocytes. In the T-PS2 thyrocytes, despite the correctly localized membrane NIS, only weak pendrin spots were seen (Fig. 4D, u–y).

Iodide uptake

NT cells showed fast iodide uptake, with cellular iodide content plateauing at 2 min and not changing over the remainder of the 30-min experiment (Fig. 5A). The kinetics curve suggests that the two iodine transporters (NIS and pendrin) were working in opposite directions. NIS initiates iodide uptake, and once intracellular membranes, NT cells express a weak band corresponding to pendrin in the process of sorting, and T-PS2 cells likewise show only a weak band. However, T-FA6 cells retain pendrin intracellularly. C, Confocal immunofluorescence images using the same pendrin antibody as in B, PS1Ab (specific for the N-terminal part of the protein). DAPI is used to show nuclei. Both a ×40 water-immersion objective with a ×3 magnification (e and g) and a ×63 oil-immersion objective with a ×1.5 magnification (f and h) were used. PS1Ab staining is localized in the Golgi (round spots beside the nucleus) and the plasma membrane (straight lines indicated by arrows) in normal thyrocytes (NT); in T-PS2 thyrocytes, pendrin appears to be retained in the Golgi (truncated proteins). D, Double immunofluorescence with NIS and PS1Ab shows intracellular and plasma membrane staining (arrows) of both proteins in NT (i–l, ×1000). Note that membrane colocalization is not frequent: the z projections obtained through stacking of confocal images show that, although localized in the membrane, the two proteins rarely coincide in the same locations, as indicated by the scarce yellow spots. T-PS2 cells (m–p, ×1000) show intracellular and plasma membrane staining of NIS, but only one isolated spot of pendrin can be seen at the plasma membrane, whereas the rest is retained in the Golgi. Similar double-immunofluorescence studies were performed using PS5Ab, specific for the C-terminal end of the pendrin protein. Both NIS and pendrin are localized at the plasma membrane in NT cells (q–t, ×1000), although both proteins seem to occupy different membrane domains (see z projections). In T-PS2 cells, the PS5Ab image is overexposed to demonstrate the absence of specific staining (u–y).
cellular iodide concentration reaches a certain level, pendrin will start efflux, maintaining the amount of iodide inside the thyrocyte at a constrained level. In contrast, T-PS2 cells showed a progressive increase in iodide level, which plateaued at around 15 min (Fig. 5A), in accordance with a single transporter (NIS) model. Vmax was two times higher in T-PS2 than NT cells, suggesting that iodide was accumulated in T-PS2 thyrocytes. In fact, the steady-state uptake after 30 min was higher in T-PS2 than NT thyrocytes (Fig. 5B).

Efflux was faster from NT than from T-PS2 cells (Fig. 5C, left). At 5 min, 40% of radioactivity had already effluxed from NT cells, but no significant efflux was seen from T-PS2; by 15 min, almost all radioactivity had effluxed from NT cells, but 40% remained in T-PS2. When residual iodide was measured at the end of the experiment, T-PS2 cells maintained higher intracellular iodide than NT cells (Fig. 5C, right).

Dose-response curves showed that after 5 min, T-PS2 cells had already reached equilibrium for iodide uptake with a Michaelis-Menten constant (Km) similar to that expected for NIS at equilibrium (22 ± 4.8 μM) (Fig. 6). In contrast, NT cells achieved equilibrium and the expected Km for iodide uptake at 1 h. Except at very short times of incubation (30 sec), when Vmax was higher for NT cells, the Vmax was twice as high in T-PS2 thyrocytes at any given time. These results suggest that normal thyrocytes behave as a complex system in which both transporters (NIS and pendrin) need to reach equilibrium slowly and that intracellular iodide concentrations are not high; however, PS-affected thyrocytes accumulate iodine through NIS, and iodine leaves the cell inefficiently through other nonspecific transporters.

**Discussion**

Two Galician families with PS were studied. SLC26A4 gene sequences showed two previously described mutations, c.279delT (8, 17) and c.578C→T (11, 16) and a novel mutation c.416–1G→A. Both families had the c.279delT mutation, and a common haplotype was seen only in c.279delT carriers, suggesting a founder effect for this mutation. Galicians have low genetic diversity in comparison with other European populations, and founder effects are not uncommon (30).

The c.416–1G→A mutation was present in family B. Although the parents denied that they were related, they were born in the same village, and a common haplotype for c.416-G→A was found in both affected and unaffected family B members but not in haplotyped members of family A. Until recently, the Galician population was organized in small and relatively isolated groups, and it is likely that the parents of propositus B have a common ancestor. The fact that this mutation has not been previously reported also suggests that c.416–1G→A originated in Galicia.

We believe that the phenotype of the A proposita (large goiter, normal serum TSH and FT₃, and hypothyroxinemia) is an adaptive response to poor organification. In experimental animals, iodine-deficient diet increases thyroid weight and favors the synthesis and secretion of T₃ resulting in an increase in serum and tissue T₃/T₄ ratio (31, 32). These changes are partially due to a TSH-independent increase in T₃ generation (32), which can lead to low serum T₃, with normal or slightly elevated T₄ and normal TSH (31). In our patient, due to the marked increase in thyroid gland size, the raised D1 and D2 levels were sufficient to maintain...
normal levels of serum FT₃. D1 and D2 will increase the intra-thyroidal conversion of T₄ into T₃, and MCT8 will maintain the transport of thyroid hormones across thyrocytes. Interestingly, the proposita’s L-thyroxine requirements were increased after thyroidectomy due to loss of the thyroid as a source of T₃. A transient increase in serum TSH in response to low thyroid hormone synthesis is a straightforward explanation for goiter development in PS patients, although other mechanisms could be involved. T-PS2 cells showed increased iodide retention leading to a steady intracellular iodide concentration. This finding suggests that intracellular accumulation of iodide may occur in thyrocytes of PS patients with adequate iodine intake, and this could have a role in the functional changes seen in diseased Pendred thyrocytes. High dietary iodine intake promotes goiter in humans (33), and although the mechanisms are not clearly defined, the Wolff-Chaikoff effect seems to play a role. However, a direct stimulating action of iodine on thyrocyte proliferation is also possible. Very high NaI concentrations (10–50 mM) over several days inhibited the proliferation of cultured rat FRTL-5 thyrocytes (34), but this was probably a toxic effect. In contrast, physiological concentrations (1 μM KI, equivalent to 150 μg/liter) stimulated basal and epidermal growth factor-induced proliferation in primary cultures of porcine follicles (35, 36) through down-regulation of intracellular CAMP levels.

Family B’s clinical phenotype is complicated by the finding of deafness with and without SLC26A4 mutations. Also, homozygotes for c.416–1G→A have congenital deafness, but not all have goiter. In fact, neither goiter nor thyroid hormone abnormalities were found in the father of the propositus, homozygous for c.416–1G→A. A similar situation has been recently reported in deaf people homozygous or compound heterozygous for mutations in SLC26A4 (5). Absence of goiter and the mild thyroid organification defect in the propositus’ father suggests that iodine can cross the apical border of thyroid cells. This can be explained by alternative splicing of the mutated mRNA, maintaining a limited amount of normal transcript. Alternatively, some iodine passage may occur through diffusion, as in the basolateral transport when NIS is absent, or another apical iodine transporter may take on pendrin’s function (37). In fact, studies in our T-PS2 thyrocytes showed that intracellular iodide was able to leave the cell, although more slowly and less efficiently than from normal NT thyrocytes.

The lack of apical pendrin immunoreactivity in the two propositus suggests that pathogenesis in our patients was due not only to functional impairment of pendrin but also to defective plasma membrane targeting (38, 39): T-PS2 thyrocytes did not express enough mature pendrin, as indicated by Western blotting and immunofluorescence, although some mature protein was pro-

![Dose-Response iodine uptake](image)

**FIG. 6.** Pendred thyrocytes quickly reach equilibrium for iodide uptake and progressively accumulate intracellular iodide. A–D, Intracellular iodide uptake by thyrocytes incubated with different NaI concentrations, at different times of incubation. Although at short incubation times (30 sec, A), the curves were similar for both lines, at 5 min (B), T-PS2 thyrocytes reached equilibrium with $K_m = 22 \pm 4.8 \mu M$, similar to the Michaelis-Menten constant at equilibrium for iodide uptake by NIS ($K_m$ around 30–40 μM). In contrast, NT showed $K_m = 81 \pm 4.2 \mu M$, far above equilibrium. Similar behavior was maintained at 30 min (C), and at 1 h (D), NT thyrocytes finally reached equilibrium ($K_m = 40.3 \pm 4.4 \mu M$). Except at very short incubation times (30 sec), when $V_{max}$ was higher in NT cells, $V_{max}$ was about twice as high in T-PS2 thyrocytes at any given time. This result suggests that initially only the NIS transporter is working in NT, as in T-PS2, but that after a few seconds pendrin starts to work in NT, and equilibrium is reached later.
duced by alternative splicing. T-PS2 cells also showed Golgi immunofluorescence, indicating retention of severely truncated proteins inside Golgi structures, as reported for other pendrin mutants in transfection studies (38, 39). Interestingly, T-FA6 cells overexpress mature pendrin, although it seems to be retained intracellularly, a finding that could be important in the pathophysiology of cold adenomas.

In conclusion, we have described two families with PS from Galicia. The founder mutation c.279delT was detected in both families. A novel mutation, c.416–1G→A, affecting SLC26A4 splicing, was also found; absence of goiter in subjects homozygous for this mutation could be explained by incomplete penetrance. Some affected subjects have goiter with normal TSH and normal thyroid hormones or hypothyroxicemia. An increase in D1 and D2 expression and activity and in MCT8 expression was found in thyroid tissue of the proposita of family A. These changes are adaptive responses to maintain a normal T3 supply at the expense of T4. No pendrin immunoreactivity was seen at the luminal border of follicles in the propositus’s thyroid glands, and T-PS2 thyrocytes showed pendrin retention in Golgi structures, indicating that mutations affect targeting of pendrin to the plasma membrane. Pendred-affected thyrocytes showed low iodide efflux and consequent accumulation, confirming the importance of pendrin as an iodide transporter.

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