The p.A2215D Thyroglobulin Gene Mutation Leads to Deficient Synthesis and Secretion of the Mutated Protein and Congenital Hypothyroidism with Wide Phenotype Variation

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Context: Thyroglobulin (TG) is a large glycoprotein and functions as a matrix for thyroid hormone synthesis. *TG* gene mutations give rise to goitrous congenital hypothyroidism (CH) with considerable phenotype variation.

Objectives: The aim of the study was to report the genetic screening of 15 patients with CH due to *TG* gene mutations and to perform functional analysis of the p.A2215D mutation.

Design: Clinical evaluation and DNA sequencing of the *TG* gene were performed in all patients. TG expression was analyzed in the goitrous tissue of one patient. Human cells were transfected with expression vectors containing mutated and wild-type human *TG* cDNA.

Results: All patients had an absent rise of serum TG after stimulation with recombinant human TSH. Sequence analysis revealed three previously described mutations (p.A2215D, p.R277X, and g.IVS30+1G>T), and two novel mutations (p.Q2142X and g.IVS46-1G>A). Two known (g.IVS30+1G/p.A2215D and p.A2215D/p.R277X) and one novel (p.R277X/g.IVS46-1G>A) compound heterozygous constellations were also identified. Functional analysis indicated deficiency in TG synthesis, reduction of TG secretion, and retention of the mutant TG within the cell, leading to an endoplasmic reticulum storage disease, whereas small amounts of mutant TG were still secreted within the cell system.

Conclusion: All studied patients were either homozygous or heterozygous for *TG* gene mutations. Two novel mutations have been detected, and we show that TG mutation p.A2215D promotes the retention of TG within the endoplasmic reticulum and reduces TG synthesis and secretion, causing mild hypothyroidism. In the presence of sufficient iodine supply, some patients with *TG* mutations are able to compensate the impaired hormonogenesis and generate thyroid hormone. **(*J Clin Endocrinol Metab* 94: 2938–2944, 2009)**

Congenital hypothyroidism (CH) is the most common endocrine disease in childhood, with a frequency of approximately 1 in 3500 live births (1). Patients with this disease can be divided into two groups: those with thyroid dysgenesis, and those with defects in any step of thyroid hormone synthesis (dyshormonogenesis) (2). Dyshormonogensis has been associated with mutations in the Na^+/I^- symporter (3), thyroglobulin (TG) (2), thyroperoxidase (4), dual oxidase 2 (5), dual oxidase mat-

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Abbreviations: AChE, Acetylcholinesterase; AU, arbitrary units; CH, congenital hypothyroidism; ER, endoplasmic reticulum; ERSD, ER storage disease; FT_4, free T_4; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; L-T_4, levothyroxine; rhTSH, recombinant human TSH; TG, thyroglobulin.

TABLE 1. Laboratory and molecular data of patients with CH bearing *TG* gene mutations

Patient no.	TSH (mU/liter)	Total T_4 (μg/dl)	FT_4 (ng/dl)	TG basal (ng/ml)	TG 48 h after rhTSH (ng/ml)	Thyroid volume[b] (ml)	^{131}Iodine uptake (%) 6 h	^{131}Iodine uptake (%) 24 h	TG mutation
I	4.15	4.8	0.63	4.2	4.0	88.0	61	82	p.A2215D
II	4.0	6.0		29.2	30.2	278	23	45	p.A2215D
III	10	4.0		9.5	12.7	212	69	70	p.A2215D
IV	4.0	7.8	0.7	5.9	6.8	20.8	70	65	p.A2215D
V	9.0	6.0	1.2	4.1	5.5	45.0	83	73	p.A2215D
VI	4.0	6.0	1.1	2.7	2.6	22.8	82	77	p.A2215D
VII[a]	0.83	9.4		0.6	0.8	12.4			p.A2215D/g.IVS30+1G/T
VIII	23.0	4.8			0.7	10.2			p.A2215D/g.IVS30+1G/T
IX	385.0			0.1		7.8	32	30.7	p.A2215D/p.R277X
X	467.1	1.1	0.23	0.8	0.5	7.4	52.6	66.4	p.R277X/g.IVS46-1G>A
XI	521.0		0.15	0.7		6.5	29.5	25.7	p.R277X
XII	759.1	1.0	0.19	0.1	0.5	5.5	14.4	25.4	p.R277X
XIII	221.3	2.9	0.41	0.1	1.9	9.6	17.4	13.8	p.R277X
XIV	571.8	1.0	0.19	0.1	0.5	7.5	48.8	57.7	p.Q2142X
XV	237.9	2.0	0.35	0.1	0.6	18.8	45.1	46.9	p.Q2142X
Normal values									
Children	0.5–4.5	4–12	0.7–1.7	1.5–15		1.6–6.0	4–18	18–32	
Adults	0.5–4.5	4–12	0.7–1.7	1.5–15	43.2 + 13	6–14	4–18	18–32	

All patients had a negative perchlorate discharge test. Patients IX–XIV: CH detected during neonatal screening.

[a] During therapy with L-T_4.

[b] As calculated by the echographic studies.

uration factor 2 (6), pendrin (7), and dehalogenase (8, 9). Dyshormonogenesis is transmitted as autosomal recessive Mendelian traits (2). The clinical spectrum of the resulting phenotypes ranges from euthyroid to mild or severe goitrous hypothyroidism (2). TG is a large glycoprotein synthesized by thyroid cells that functions as a matrix for thyroid hormone synthesis. The *TG* gene is located on chromosome 8q24 (10). It consists of 48 exons and is responsible for encoding a protein with 2768 amino acids (10). Eighty percent of the monomer primary structure is characterized by the presence of three types of repetitive units (11, 12). The remaining 20%, which constitutes the carboxy-terminal domain of the molecule, is not repetitive, shows a striking homology with acetylcholinesterase (AChE), and may function as an intramolecular chaperone (13–15). So far, 39 inactivating mutations have been identified and characterized in the human *TG* gene, consisting of 23 missense, five nonsense and eight splice-site mutations, two single nucleotide deletions, and one nucleotide insertion (2, 16, 17).

In this study, we report the functional analysis of the previously described p.A2215D (18) mutation found in Brazilian patients with CH with detectable serum TG but with no response to stimulation with recombinant human TSH (rhTSH). This mutation promotes deficient synthesis of TG mRNA and reduction of TG secretion and results in an endoplasmic reticulum storage disease (ERSD). Mutated TG molecules are able to escape from the endoplasmic reticulum (ER) and synthesize small amounts of thyroid hormone. This may, in part, explain why some of the CH patients bearing this mutation present with euthyroidism. We also report the genetic screening of 15 patients with CH secondary to defective synthesis of TG and the identification of two novel mutations in the *TG* gene, p.Q2142X and g.IVS46-1G>A; three previously described mutations, p.A2215D, p.R277X, and g.IVS30+1G>T; a novel compound heterozygous constellation, p.R277X/g.IVS46-1G>A and p.A2215D/p.R277X (18).

Patients and Methods

Patients

Patient I

Patient 1 was a female diagnosed with CH at the age of 26 yr (Table 1). Ultrasound examination revealed an 88-ml goiter with multiple nodules. According to the patient, neck enlargement has been present since the age of 8 yr. The patient has four children in whom CH has been excluded by clinical and laboratory tests. Four of her five siblings also have an enlargement of the thyroid. The patient underwent total thyroidectomy with histological diagnosis of an adenomatous goiter and follicular hyperplasia with Hurthle cells. Her parents were consanguineous (first-degree cousins).

Patient II

Patient II, a male, was diagnosed with congenital goiter with a large goiter (278 ml) when he was 34 yr old (Table 1). At the age of 10 yr, this patient underwent partial thyroidectomy. Due to goiter regrowth, total thyroidectomy was performed, and histological diagnosis was follicular hyperplasia.

Patient III

Patient III, a brother of patient II, was diagnosed with a large congenital goiter (212 ml) at the age of 27 yr (Table 1). He underwent total thyroidectomy, and the histological diagnosis was microfollicular hyperplasia. Both he and his brother presented an enlarged thyroid since early childhood. Their parents were not consanguineous.

Patient IV

Patient IV, a male, was diagnosed with CH at the age of 2 months, and treatment with levothyroxine (L-T_4) was started (Table 1). He had an

enlarged thyroid since early childhood. At the age of 11 yr, this patient presented with delayed bone age (10 yr) and a euthyroid goiter (20.8 ml) Total thyroidectomy was performed at the age of 16 yr. His parents were not consanguineous.

Patient V

Patient V, a male, was diagnosed with goitrous CH at the age of 9 yr (Table 1). At that time, bone age was 5 yr, thyroid volume on ultrasound was 45 ml, and treatment with L-T_4 was initiated.

Patient VI

Patient VI, the sister of patient V, was diagnosed with CH when she was 4 yr old. At that chronological age, her bone age was 2.6 yr. She presented with a thyroid enlargement of 22.8 ml on ultrasound and was treated with L-T_4. Parents of patients V and VI were consanguineous.

Patients VII and VIII

Clinical history of these two siblings has been previously described (19, 20).

Patients IX, X, XI, XII, XIII, and XIV

These four males and two females were diagnosed with CH during neonatal screening which was confirmed at the age of 3 yr. Their parents were not consanguineous.

Patient XV

Patient XV, the sister of patient XIV, was diagnosed with CH at the age of 6 yr, when she was started on L-T_4. This patient presented with mild mental retardation. Parents of patients XIV and XV were not consanguineous.

This study was approved by the Ethical Committee of the Hospital das Clínicas, University of São Paulo Medical School. Informed consent was obtained from the parents of the patients.

Thyroid function tests

Serum total T_4, free T_4 (FT_4), total T_3, TSH, and TG levels were determined by electrochemiluminescence immunoassay (Roche Corporation, Indianapolis, IN). ^{131}Iodine uptake was performed at 6 and 24 h. Perchlorate discharge test was negative in all patients. Thyroid volume was calculated after echographic studies.

rhTSH test

Thirteen affected patients received 0.1 mg of im rhTSH to test for defective synthesis and secretion of TG. Serum TG values were measured at baseline and at 24 and 48 h after rhTSH injection (21).

DNA sequencing

Peripheral blood DNA from all patients was isolated by the sodium dodecyl sulfate-proteinase K method. The complete coding sequence of the human *TG* gene, including splice sites and flanking intronic regions of each intron, was amplified using primers and conditions reported previously (22). DNA sequencing of each amplified fragment was performed with an ABI 377 system (Applied Biosystems Corp., Foster City, CA) using the same *TG*-specific primers used in the amplification step. The sequences were compared with those of the human *TG* gene sequence (GenBank accession no. NT_008046). The amino acid numbering does not include the 19 amino acid of the signal peptide. A control group of 200 healthy subjects without thyroid disease was evaluated to determine whether the observed DNA substitutions were *bona fide* mutations or polymorphisms. All control subjects had normal FT_4 (0.7–1.5 ng/dl) and TSH levels (0.5–4.5 μU/ml).

Tissue sample

A sample of the thyroid tissue was collected from patient I during total thyroidectomy. One fragment of the specimen was immediately frozen in liquid nitrogen. Others were kept in formalin for immunohistochemistry and in 2.5% glutaraldehyde for electron microscopy. Normal thyroid tissue from a patient who had undergone surgery due to a solid nodule was used as control. A control sample was collected from the opposite thyroid lobe.

Quantification of TG mRNA by real-time PCR

Total RNA was isolated using TRIzol LS (GIBCO BRL, Life Technologies, Gaithersburg, MD), and cDNA was synthesized using 250 ng of RNA and Super Script III Reverse Transcriptase (Invitrogen, Carlsbad, CA). Expression levels of TG and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (internal control) mRNA were quantified by real-time PCR assays using ABsolute QPCR SYBR Green Mix (ABgene, Surrey, UK) on a Rotor-Gene 3000 (Corbett Research, Mortlake, Australia). The intron-spanning primers were: TG forward, GAGCCCTACCTCTTCTGGCA; TG reverse, GAGGTCCTCATTCCTCAGCC (23); GAPDH forward, GCTGGCATTGCCCTCA; and GAPDH reverse, GGCAGGGACTCCCCAG (24). The patient sample was assayed in triplicate and 10 normal thyroid control samples in duplicate. The reaction mixture was denatured for 20 sec at 95 C and subjected to 40 cycles of 30 sec at 62 C and 30 sec at 72 C. The PCR efficiency was similar for both genes ($E \approx 1$); consequently, the DDCT method (ABI Prism 7700 Sequence Detection System; Applied Biosystems, User Bulletin no. 2) was used to calculate gene expression, which is reported as relative arbitrary units (AU).

Construction of human *TG* cDNA expression vectors

Several overlapping fragments of the human *TG* coding sequence were generated by RT-PCR using proof reading *Pfu* polymerase (Invitrogen). The fragments were joined by overlap extension PCR or using unique restriction sites in the *TG* coding sequence. The final construct was inserted into a pcDNA3.1 plasmid (Invitrogen) and submitted to direct sequence analysis. The *TG* coding sequence contains at least 15 polymorphisms. We chose the human *TG* sequence reported by van de Graaf *et al.* (12) as a reference sequence and corrected any deviation from this sequence using site-directed mutagenesis. The mutation in position 6701 caused by a cytosine to adenine transition (c.6701C>A; p.A2215D) (18), identified in patient I was introduced into the TG cDNA using QuikChange XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA).

Cell culture

HEK293 and TSA201 cells were cultured at 37 C in a 5% CO_2 atmosphere in DMEM with high glucose, L-glutamine, pyridoxine hydrochloride (Invitrogen, Life Technologies), supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen, Life Technologies).

Transient transfections

HEK293 and TSA201 cells were transiently transfected with pcDNA3.1 (Invitrogen, Life Technologies) expression vectors encoding full-length cDNA of wild-type (pTGWT) or mutated *TG* (pTGA2215D) with the Ca_2Cl method (25). Nontransfected cells were used as negative controls. Twenty-four hours after transfection, the first cell culture medium was collected, and 2 ml of DMEM were added. At 48 h, media were collected again, and cells were lysed to obtain total protein extracts.

Quantification of TG protein

TG concentrations were determined in the supernatants collected 24 and 48 h after seven independent experiments of transfection of the pTGWT and pTGA2215D vectors in HEK293 cells with a DELFIA Thyroglobulin (hTg) kit (DELFIA, Wallac, Oy, Turku, Finland). Cotransfection of a pGL3 basic plasmid (Promega, Madison WI) for determi-

nation of transfection efficiency by luciferase activity was performed to normalize the results.

Western blot analysis

Thyroid tissue extracts and total protein extracts (10 μg) from transient transfection were analyzed by reducing SDS-PAGE (5% gel), followed by Western blotting. For immunoblotting, proteins were transferred to nitrocellulose membranes and probed with antibodies against TG, followed by enhanced chemiluminescence (ECL; Amersham Bioscience, Piscataway, NJ). A rabbit polyclonal antihuman TG (DakoCytomation, Glostrup, Denmark) and a mouse monoclonal antihuman TG (DakoCytomation) were used as primary antibodies. Peroxidase-conjugated monoclonal antirabbit and antimouse antibodies were used as secondary antibodies (Bio-Rad Laboratories, Inc., Hercules, CA; and DakoCytomation, respectively). Reducing SDS-PAGE (14% gel) followed by immunoblotting probed with mouse monoclonal antiactin (Sigma-Aldrich, St. Louis, MO) was performed as expression control. The immunoblots were analyzed by scanning densitometry

Histological analysis

Thyroid tissue samples from patient I were stained with hematoxylin-eosin and analyzed by immunohistochemistry with a rabbit polyclonal antihuman TG antibody and electron microscopy.

Results

rhTSH stimulation

Abnormal TG synthesis and secretion were confirmed by near absence of serum TG elevation 24 and 48 h after stimulation with rhTSH in the patients with CH (Table 1) compared with normal controls.

TG mutations

In these patients, sequence analysis of the *TG* gene revealed three previously described mutations (p.A2215D, p.R277X, and g.IVS30+1G>T) (18, 26, 27) and two novel mutations (p.Q2142X and g.IVS46-1G>A). The p.A2215D mutation, which replaces alanine with an aspartic acid, was found on both alleles in patients I, II, III, IV, V, and VI and on one allele in three other patients (VII, VIII, and IX). Patients VII and VIII harbored the known mutation g.IVS30+1G>T and were found to be compound heterozygous for g.IVS30+1G>T/p.A2215D (19). Patient IX also harbored the previously described p.R277X mutation resulting in a compound heterozygous constellation (p.A2215D/p.R277X) (18). The previously described p.R277X mutation, which replaces arginine with a stop codon, was found in homozygosity in patients X, XI, XII, and XIII. One of the novel mutations was caused by a cytosine to thymine transition located at nucleotide 6481 (c.6481 C>T) in exon 37 and replaces a glutamine in codon 2142 with a stop codon (p.Q2142X). This mutation was found in both alleles in siblings XIV and XV. The other novel mutation was caused by a guanine to adenine transition in position −1 (receptor splice site) of intron 46 (g.IVS46-1G>A). This mutation was found in one allele of patient X, who also harbored the previously described p.R277X mutation. This generated a new compound heterozygous constellation (p.R277X/g.IVS46-1G>A). The new mutations were not found in the genome of 200 subjects without thyroid disease (normal controls).

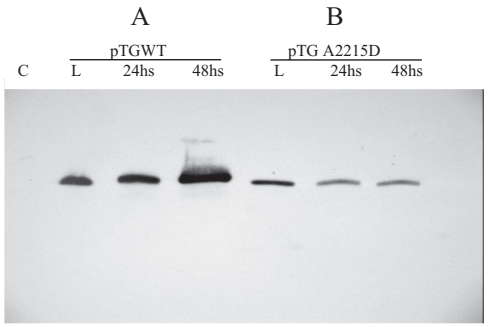

FIG. 1. Immunoblot with a TG monoclonal antibody with 10 μg of total protein from cell lysate and supernatants after transfection with wild-type *TG* (pTGWT) (A); and mutated *TG* (pTG A2215D) (B). C, Negative control; L, total protein from cell lysate; 24 and 48 h, total protein from supernatants collected at 24 and 48 h.

TG expression in transient transfections

Western blotting experiments revealed that the wild-type TG protein was more abundant in the supernatant collected at 48 h compared with TG concentrations found in cell lysates and in supernatants collected 24 h after transfection. By contrast, the mutant TG protein was more abundant in cell lysates than in supernatants (Fig. 1). These results suggest that the p.A2215D mutation allows the synthesis of the mutant TG protein, but that it is retained intracellularly, causing a deficiency of TG secretion. This hypothesis was confirmed by quantification of TG concentrations in the supernatants of transfected cells. In supernatants collected 24 h after transfection, the levels of mutated TG were undetectable, whereas wild-type TG concentration (mean ± SD) was 7.50 ± 3.3 ng/ml. The difference in TG expression levels was also detected 48 h after transfection (mutated TG levels, 0.19 ± 0.03 ng/ml; and wild-type TG levels, 88.13 ± 35.07 ng/ml).

TG analysis in CH thyroid tissue

We analyzed the total TG protein present in thyroid gland homogenates of patient I and normal control. As shown in Fig. 2, patient I exhibited a stainable band of a size similar to the normal TG, but with reduced amount (nine times) when compared with the normal control after normalization with actin expression.

Thyroid tissue TG mRNA quantification

In patient I, the mean ± SD concentration of TG mRNA in thyroid tissue samples was 1648.9 ± 168.7 AU. These levels were 3.68 times lower compared with levels found in samples from 10 normal controls (6062.9 ± 1308.3 AU) (Fig. 3).

Immunonohistochemical localization of TG

Immunohistochemical localization of TG showed a marked decrease of immunopositivity in the follicular lumina of thyroid sections from patient I when compared with a normal control. Remarkably, however, immunopositivity for TG was clearly demonstrable in the thyroid epithelium of the affected patient (Fig. 4, A1 and A2). These results are in agreement with hematoxylin-eosin staining of the goitrous tissue that clearly indicated absence of colloid (TG storage) and very large follicular lumina (Fig. 4, B1 and B2).

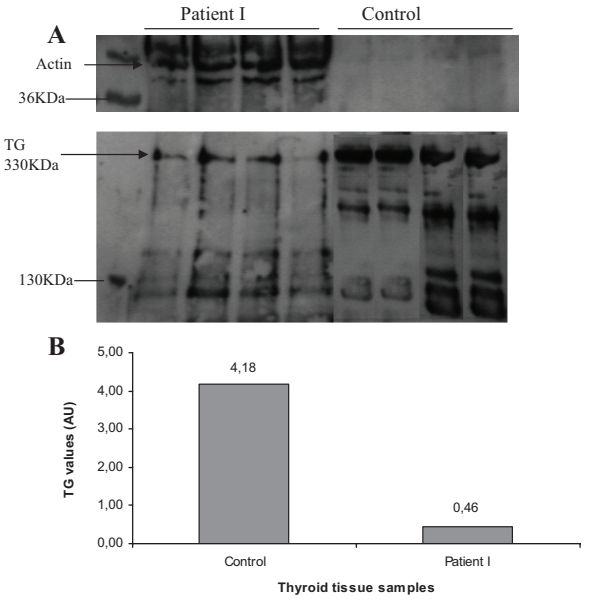

FIG. 2. A, Hybridization with specific antibodies to TG and actin. Patient I, Protein extracts from thyroid tissue samples of patient I bearing the p.A2215D TG mutation. Control, Protein extract from thyroid tissue samples of a subject without thyroid disease. B, TG values after normalization with actin.

Electron microscopy

Electron microscopy of thyroid tissue samples from patient I revealed prominent vesicular organelles, identified as dilated ER. In contrast, normal tubular ER was seen in control samples (Fig. 5, A and B).

Discussion

A genetic screening of 15 patients with CH due to possible *TG* gene defects allowed the identification of two new mutations (p.Q2142X and g.IVS46-1G>A), three known mutations (p.A2215D, p.R277X, and g.IVS30+1G>T) (18, 26, 27), and two compound heterozygous constellations (p.A2215D/p.R277X and p.R277X/g.IVS46-1G>A). The new g.IVS46-1G>A was identified on one allele in patient X, who also harbored the previously described mutation p.R277X, resulting in a novel compound heterozygous constellation (g.IVS46-1G>A/p.R277X). This intronic mutation may not be considered a polymorphism because it could not be detected in subjects without thyroid disease. The functional consequences of this new mutation could be the loss of exon 47 due

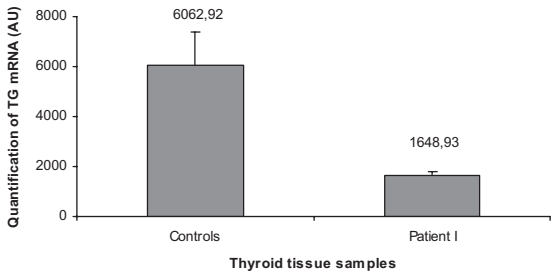

FIG. 3. Quantification of TG mRNA (mean ± SD) in thyroid tissue samples from patient I and 10 control subjects.

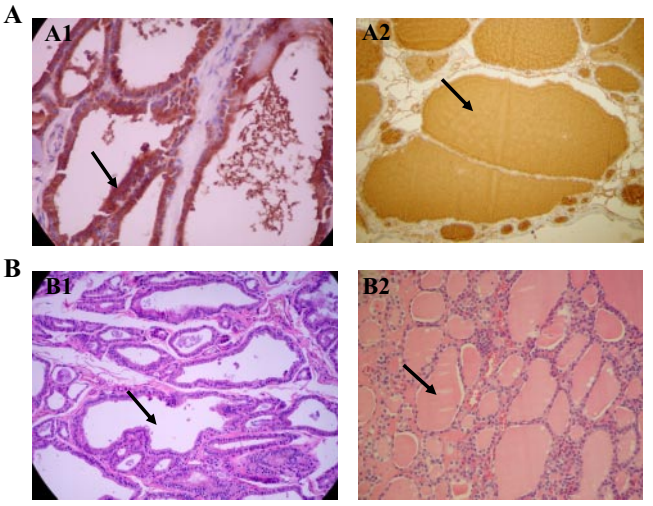

FIG. 4. A, Localization of TG by immunohistochemistry. A1, Thyroid tissue sample from patient I. The *arrow* shows positivity for TG inside the cell. A2, Normal control tissue. Note the abundance of TG in the follicular lumen. B, Hematoxylin-eosin staining. B1, Thyroid tissue sample from patient I. The *arrow* shows absence of TG within the colloid. B2, Normal control tissue. The *arrow* shows presence of TG within the colloid.

to an aberrant splicing and the retention of the TG protein inside the ER due to a structural alteration, as already observed in another intronic mutation identified in the *TG* gene (28). The other novel mutation, p.Q2142X, was identified in a homozygous condition in two siblings (patients XIV and XV); it was not detected in 200 subjects without thyroid disease. This mutation is localized in the TG type III repeat domain, which is associated with glycosylation, an essential process that allows the migration of the TG from the ER to the Golgi (28). According to Park and Arvan (14), the presence of a native AChE-like region that functions as a dimerization domain is important for TG folding and maturation that leads to escape from ER quality control. Recently, Lee *et al.* (15) proposed that as long as the AChE domain is itself properly folded, it functions as an intramolecular chaperone within the context of full-length TG, facilitating the efficiency of "on-pathway" protein folding, leading to ER exit. The truncated p.Q2142X form of TG still harbors both the acceptor tyrosine 5 (exon 2) and the donor tyrosine 130 (exon 4) of the amino-terminal hormonogenic domain. This premature stop codon eliminates the AChE-like region and the carboxyl-terminal hormonogenic domain. Recently, the introduction of a stop codon in mouse TG residue cysteine 175 showed that the

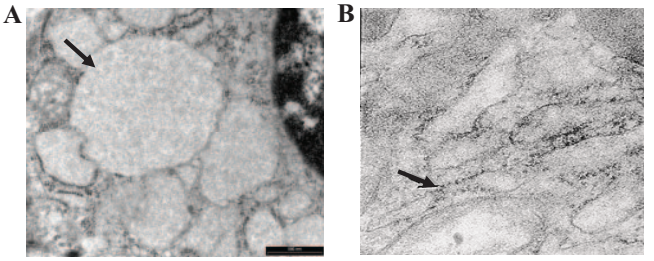

FIG. 5. Localization of TG by electron microscopy. A, Thyroid tissue sample from patient I. The *arrow* shows a marked dilatation of the ER. B, Normal control tissue. The *arrow* shows a normal ER.

p.C175X mutant was secreted as a truncated TG protein (17). This strongly suggests that the secretory pathway continues to work despite the complete deletion of AChE-like region (15). Functional analysis of this mutation would help to make it clear whether the truncated protein is secreted.

In the present study, we performed a functional analysis of the previously described mutation p.A2215D, identified as a homozygous mutation in six patients and in heterozygous form in three other individuals. Alignment analysis of this mutation revealed that the wild-type alanine residue at position 2215 is strictly conserved in all TG and AChE specimens analyzed (18). Some authors have documented that CH in *cog/cog* mice and *rdw* rats is caused by a missense mutation in the AChE-like domain of the TG molecule (29, 30). Both mutated TG proteins exhibit a severe defect in the exit from the ER, causing a thyroidal ERSD. A cell culture model performed in the present study suggests that the mutation p.A2215D results in deficient TG secretion with retention of the molecule within the cell (Fig. 1). Quantitative measurement of secreted TG confirmed this hypothesis. The immunohistochemical localization of TG in the thyroid tissue of patient I showed that this mutated protein is indeed retained within the cell, more precisely within the ER. Analysis of the TG synthesis in thyroid tissue samples from patient I revealed that the amount of protein was nine times lower when compared with samples from a normal control. A similar result was observed by Hishinuma *et al.* (31) in a patient with the p.C1977S mutation in the *TG* gene. Baryshev *et al.* (32) have proposed two different explanations for these results. One was that such a decrease would occur due to augmented susceptibility of structurally destabilized TG to proteolysis. However, the authors failed to detect immunoreactive proteolytic fragments to prove this point. Another explanation would be that in patients with ERSD due to *TG* gene mutations, the protein could form aggregates with molecular chaperones and not be detected by conventional SDS-PAGE. However, TG mRNA levels were not investigated in their report. In the present study, we detected that the TG mRNA levels were 3.68 times higher in the control than in thyroid tissue of the affected patient, suggesting either a reduction in the *TG* transcription or an increase of the mRNA degradation in the hypothyroid tissue.

Despite the retention in the ER, some mutated TG molecules may escape from the ER and migrate to the colloid, allowing synthesis of small amounts of thyroid hormone. This observation provides at least a partial explanation for the euthyroidism found in some of our patients. This would explain the finding of serum TSH, total T_4, FT_4, and TG levels closer to normal in the absence of L-T_4 treatment in some homozygous patients for the p.A2215D (Table 1). This may be indicative that more of the mutant TG is functionally active in the presence of adequate nutritional iodine intake. In contrast, the homozygous patients (XI, XII, and XIII) with the p.R277X and g.IVS30+1G>T TG mutations had elevated serum TSH and undetectable TG levels. Previous studies have suggested that the p.R277X TG mutation may allow the synthesis of T_4 in the amino terminal past of the small TG fragment (33, 34). However, patients harboring this mutation present with severe CH. Similarly, the g.IVS30+1G>T TG is almost completely retained in the ER and associated with severe deficiency of thyroid hormone already in prenatal life and leading to fetal goiter (19). Patients VII, VIII, and IX who were heterozygous for the p.A2215D mutation had very high serum TSH values and undetectable TG levels before and after rhTSH stimulation. This phenotype may be the consequence of the presence of only one copy of the p.A2215D mutation along with another mutation, whereas phenotypic variations among patients with the same mutations have been previously reported.

In conclusion, all studied patients were either homozygous or heterozygous for inactivating *TG* gene mutations. Two novel mutations have been detected, and we show that the TG mutation p.A2215D promotes the retention of TG within the ER and reduces its synthesis and secretion to the colloid, causing decreased hormonal synthesis and mild hypothyroidism.

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