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Review

Genetics and phenomics of hypothyroidism and goiter due to thyroglobulin mutations

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ABSTRACT

Thyroglobulin (TG) defects due to TG gene mutations have an estimated incidence of approximately 1 in 100,000 newborns. This dyshormonogenesis displays a wide phenotype variation and is characterized usually by: the presence of congenital goiter or goiter appearing shortly after birth, high ^{131}I uptake, negative perchlorate discharge test, low serum TG and elevated serum TSH with simultaneous low serum T_4 and low, normal or high serum T_3. Mutations in TG gene have been also reported associated with endemic and euthyroid nonendemic simple goiter. TG gene defects are inherited in an autosomal recessive manner and affected individuals are either homozygous or compound heterozygous for mutations. Up to now, 50 mutations have been identified and characterized in the human TG: 23 missense mutations, 10 nonsense mutations, 5 single and 1 large nucleotide deletions, 1 single nucleotide insertion and 10 splice site mutations. The functional consequences of this mutations could be structural changes in the protein molecule that alter the normal protein folding, assembly and biosynthesis of thyroid hormones, leading to a marked reduction in the ability to export the protein from the endoplasmic reticulum.

Contents

1. Introduction

Thyroglobulin (TG) is the most abundant expressed protein in the thyroid gland, secreted by thyrocyte into the follicular lumen by exocytosis (Dunn and Dunn, 2000). The thyroid cells produce free thyroid hormones by proteolytic cleavage of the TG, which are delivered to the blood circulation for action at their peripheral target tissues. TG synthesis occurs exclusively in the thyroid gland. In this respect, the promoter region of the TG gene is activated by three transcription factors, thyroid transcription factor 1 (TTF-1, also known as TITF1, NKX2.1 or T/EBP) (Javaux et al., 1992), thyroid transcription factor 2 (TTF-2, also known as TITF2, FOXE1 or FKHL15) (Zannini et al., 1997) and paired box transcription factor 8 (PAX8) (Zannini et al., 1992). Although none of these transcription factors are expressed only in the thyroid, their combination is unique to this gland (Damante et al., 2001). TG functions as the matrix for thyroid hormones synthesis and for the storage of the inactive form of thyroid hormones and iodine. Biosynthesis of thyroid hormones is critically dependent upon the native three-dimensional structure of TG. The TG has been proposed as a transcriptional regulator on thyroid-specific genes (Suzuki et al., 1998) and more recently, as a direct growth regulator of thyroid epithelial cells (Hayashi et al., 2009). The study of TG is of great relevance to thyroid pathophysiology. Mutations in the human TG gene have been reported and are associated with congenital goi-

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ter with hypothyroidism or euthyroidism (Medeiros-Neto et al., 1993; Rivolta and Targovnik, 2006) and endemic and euthyroid nonendemic simple goiter (Corral et al., 1993; Pérez-Centeno et al., 1996; González-Sarmiento et al., 2001). The TG gene has also been identified as the major susceptibility gene for familial autoimmune thyroid disease (AITD), by linkage analysis using highly informative polymorphic markers (Tomer et al., 2002).

The present review provides an overview of the most recent developments on the mutational processes and pathogenesis mechanisms responsible for dyshormonogenesis due to defective TG synthesis.

2. Thyroglobulin and the biosynthesis of thyroid hormones

Differentiated thyroid follicular cells are characterized by the ability to synthesize the thyroid hormones, the 3,5,3′-triiodothyronine (T_3) and 3,5,3′,5′-tetraiodothyronine or thyroxine (T_4). This process requires the presence of iodide, thyroperoxidase (TPO), a supply of H_2O_2 and an iodine acceptor protein, TG (Fig. 1) (Taurog, 2000). The thyroid gland maintains a concentration of free iodide 20–40 times higher than that of plasma under physiological conditions. The iodide transport is a two steps process involving transporters located either in the basal or apical membranes (Fig. 1). Iodide is accumulated from the blood into the thyroidal cell through the sodium/iodide symporter (NIS) localized in the basolateral membrane (Dohán et al., 2003). On the other hand, pendrin (PDS, also known as SLC26A4), located in the apical membrane, is responsible for the iodide transport from epithelial cell to follicular lumen (Kopp et al., 2000). At the cell apex, TPO is the enzyme responsible: (1) for oxidizing I^- into I^+, (2) iodinating tyrosyl groups of TG resulting in 3-monoiodotyrosine (MIT) and 3,5-diiodotyrosine (DIT) and (3) binding these groups to form hormonal molecules T_3 and T_4 (Fig. 1) (Taurog, 2000). TPO is composed of a large extracellular domain, a transmembrane domain and a short intracellular tail (Libert et al., 1987; Kimura et al., 1989). H_2O_2 is used as a substrate by TPO in the organification of iodide. The H_2O_2 generation system of the thyroid involves a duox system composed of dual oxidase 1 (DUOX1, also known as ThOX1, LNOX1), dual oxidase 2 (DUOX2, also known as ThOX2, LNOX2), dual oxidase maturation factor 1 (DUOXA1) and dual oxidase maturation factor 2 (DUOXA2) (Dupuy et al., 1999; De Deken et al., 2000; Grasberger and Refetoff, 2006). The DUOXA1 and DUOXA2 are endoplasmic reticulum (ER) resident proteins which are responsible of ER-to-Golgi transition, maturation, and translocation to the plasma membrane of DUOXs (Grasberger and Refetoff, 2006).

The mature TG molecules containing MIT, DIT, T_4 and T_3 remain in the lumen of thyroid follicles (Fig. 1). Thyroid gland produces predominantly T_4 together with a small amount of T_3. Afterwards, TG is subjected to proteolysis, MIT and DIT are subsequently deiodinated by the specific iodotyrosine dehalogenase 1 (DEHAL1) (Fig. 1) (Gnidehou et al., 2004). Most T_3 is produced by enzymatic deiodination of T_4 in peripheral tissues. Three enzymes have been identified, called Type-1, Type-2 and Type-3 iodothyronine deiodinases, they form a family of homologous selenoproteins (Bianco et al., 2002).

3. Classification of congenital hypothyroidism

Primary CH affects about 1 in 3000 newborns and is characterized by elevated levels of thyroid-stimulating hormone (TSH) resulting from reduced thyroid function. This disease is caused

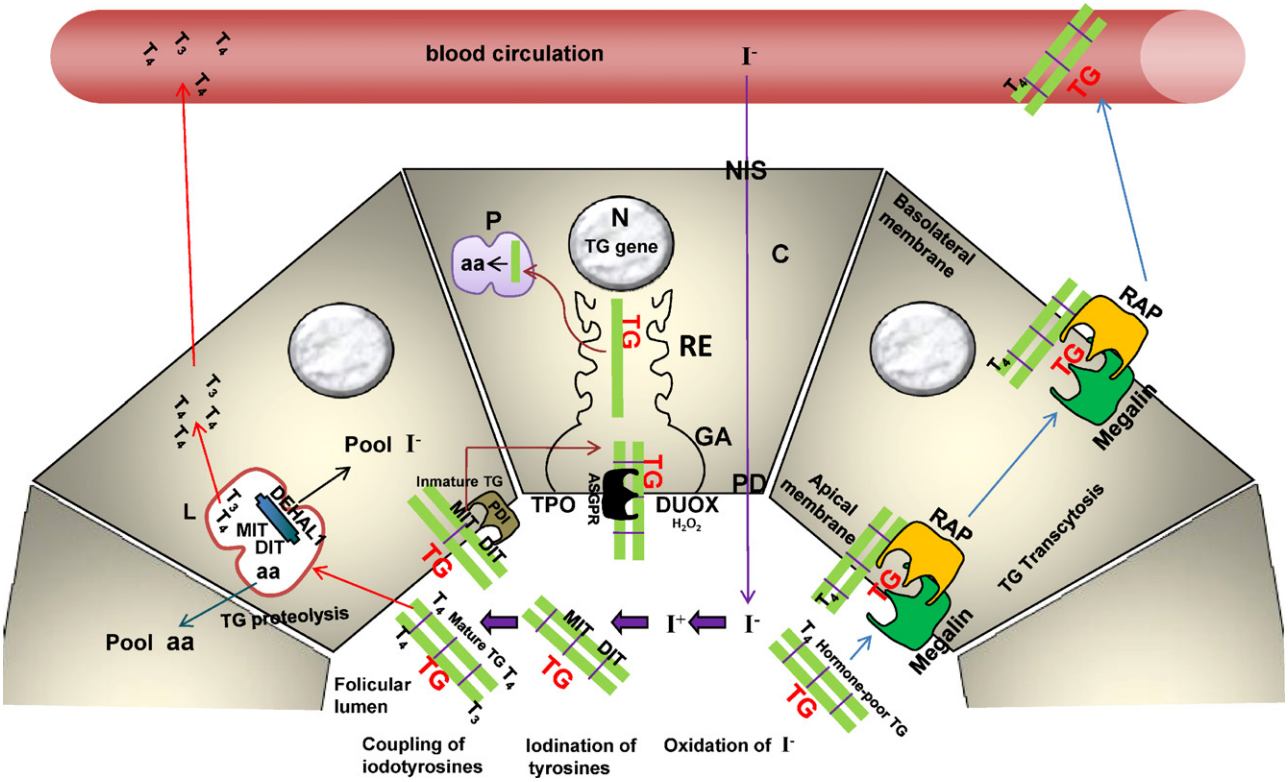

Fig. 1. Schematic representation of the maturation, internalization and transepithelial transport pathways of thyroglobulin in thyrocyte. The relevant processes in the different organelles (N, nucleus; C; cytoplasm; RE, rough endoplasmic reticulum; GA, Golgi apparatus; P, peroxisome; L, lysosome) and thyroid-specific proteins (TG, thyroglobulin; TPO, thyroperoxidase; NIS, sodium iodide symporter; PD, pendrin; DUOX, dual oxidase; DEHAL1, iodotyrosine deiodinase) are shown. In the follicular lumen oxidation of iodine, iodination of tyrosines (MIT, 3-monoiodotyrosine; DIT, 3,5-diiodotyrosine) and coupling of iodotyrosines takes place on tyrosine residues in TG, resulting in 3,5,3′-triiodothyronine (T_3) and 3,5,3′,5′-tretraiodothyronine (T_4) synthesis. The asialoglycoprotein receptor (ASGPR) transports new synthesized TG to the follicular lumen. Whereas, the protein disulfide isomerase (PDI) would recognize immature TG for recycling through the GA and megalin and LDL receptor-associated protein (RAP) would interact with TG for apical to basolateral transcytosis. aa, amino acids.

by disorders of thyroid gland development (dysembriogenesis), which accounts for 80–85% of cases (De Felice and Di Lauro, 2004), or by defects in any step of thyroid hormone synthesis (dyshormonogenesis), which accounts for remaining 15–20% of cases. Thyroid dyshormonogenesis has been linked to mutations in the NIS (Fujiwara et al., 1997; Dohán et al., 2003), pendrin (Everett et al., 1997; Kopp, 2000), TPO (Abramowicz et al., 1992), DUOX2 (Moreno et al., 2002), DUOXA2 (Zamproni et al., 2008), DEHAL1 (Moreno et al., 2008; Afink et al., 2008) and TG genes (Medeiros-Neto et al., 1993; Rivolta and Targovnik, 2006) (Fig. 2). These mutations produce a heterogeneous spectrum of congenital goitrous hypothyroidism, with an autosomal recessive mode of inheritance. In untreated patients CH can result in abnormal growth and development as well as of severe mental retardation. Whereas, early diagnosis and treatment with L-thyroxine as a consequence of the neonatal screening programs led to normal development in nearly all cases.

Perchlorate discharge test and the measurement of TG serum concentration represented an important diagnostic tool that helps to differentiate patients with iodide organification disorder of those with DEHAL1 or TG deficiencies.

Iodide organification defects are associated with mutations in the TPO, DUOX2, DUOXA2 and PDS genes and characterized by a positive perchlorate discharge test, indicating a defect in converting accumulated iodide to organically bound iodine (Fig. 2). This type of congenital hypothyroidism is characterized by intact iodide trapping and normal or elevated serum TG and subdivided into total iodide organification defects (TIOD) and partial iodide organification defects (PIOD), depending on the percent radio-iodide discharged from the thyroid gland by perchlorate. Mutations in PDS gene cause Pendred syndrome characterized by congenital sensorineural hearing loss and goiter with or without hypothyroidism. Most affected individuals remain euthyroid, although serum thyroglobulin levels may be elevated and perchlorate discharge test positive (Everett et al., 1997).

Patients with iodotyrosine dehalogenase deficiency will also develop goiter with hypothyroidism, when dietary iodide is limiting. Recently, the first mutations in the DEHAL1 gene have been reported (Moreno et al., 2008; Afink et al., 2008). In this patient evaluated with a perchlorate discharge test, there was no increased release of radioiodine after administration of the competitor, indicating that the organification process itself was not affected (Fig. 2), whereas the serum TG levels were elevated. In contrast, the presence of low TG level and also negative perchlorate discharge test in a goitrous individual suggest a TG defect (Fig. 2) (Medeiros-Neto et al., 1993; Rivolta and Targovnik, 2006).

Finally, patients with an iodide transport defect by mutations in NIS gene have a normal-sized or somewhat enlarged thyroid gland

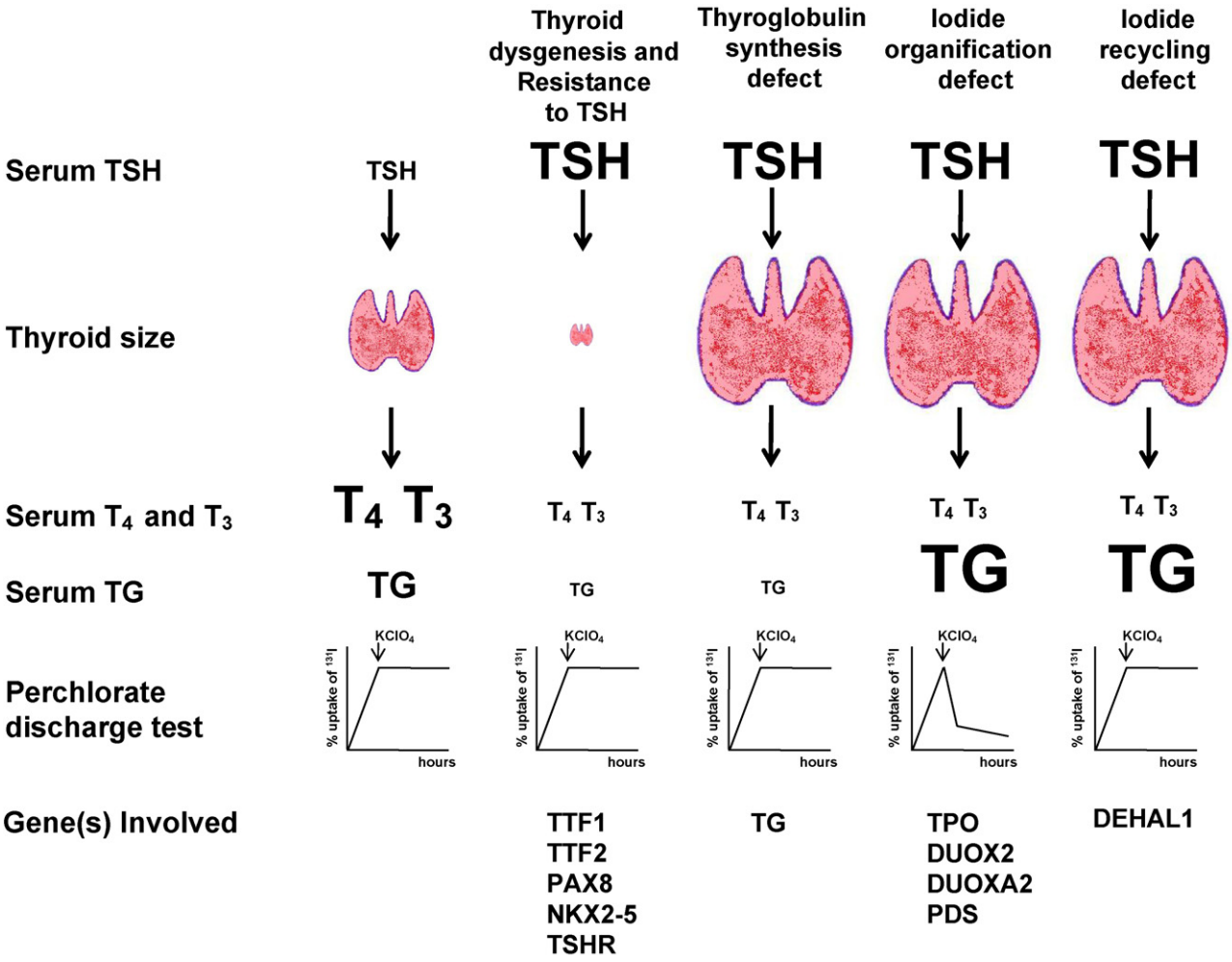

Fig. 2. Thyroid size and biochemical parameters of patients with congenital hypothyroidism due to dysembryogenesis (thyroid dysgenesis and resistance to TSH) and dyshormonogenesis (thyroglobulin synthesis, iodide organification and iodide recycling defect).
TG, thyroglobulin; TPO, thyroperoxidase; TSH, thyrotropin, TSHR, receptor for TSH; TTF-1, thyroid transcription factor 1; TTF-2, thyroid transcription factor 2; PAX-8, paired box transcription factor 8; NKX2-5, cardiac transcription factors; DUOX2, dual oxidase 2; DUOXA2, dual oxidase maturation factor A2; PDS, pendrin; DEHAL1, iodotyrosine deiodinase 1.

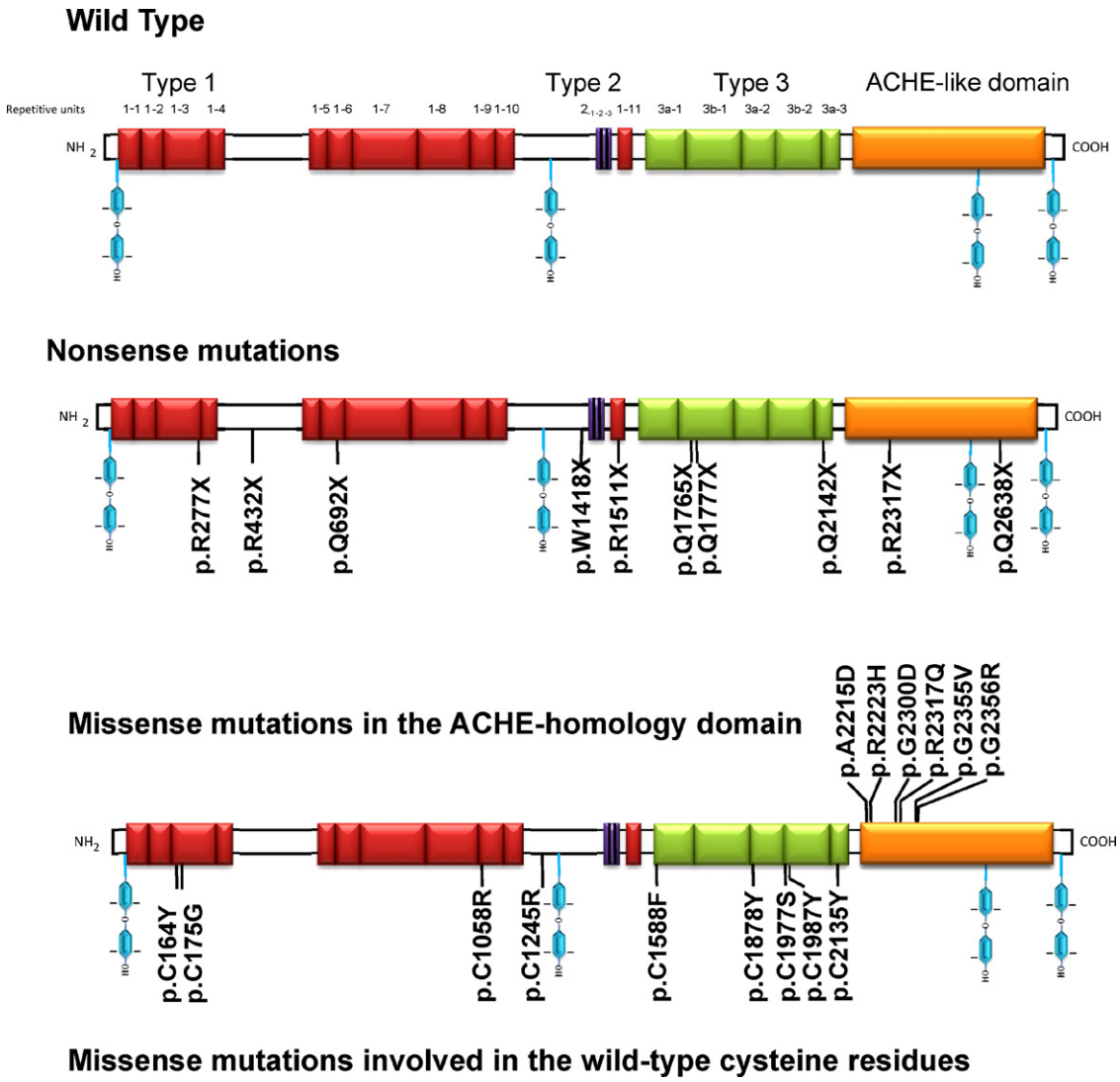

Fig. 3. Structural organization of the wild-type and mutant of thyroglobulin proteins due to nonsense mutations and missense mutations. The repetitive units of Type 1, 2 and 3 and the acetylcholinesterase-homology domain (ACHE-like domain) are represented by boxes. The N-terminal limit of repeat Type 1-5 is ambiguous. The positions of T_4 and T_3 are shown.

by ultrasonography, elevated plasma TG levels and no radio-iodide uptake (Fujiwara et al., 1997; Dohán et al., 2003).

4. Thyroglobulin gene: structure and expression

TG is a large homodimeric secretory protein of 660 kDa with a high degree of glycosylation. In human, it is coded by a single copy gene, 270 kbp long (van Ommen et al., 1983; Targovnik et al., 1984, 1992a; Christophe et al., 1985; Baas et al., 1986; Parma et al., 1987; Mendive et al., 1999, 2001; Moya et al., 2000) that maps on chromosome 8q24.2–8q24.3 (Brocas et al., 1985; Avvedimento et al., 1985; Baas et al., 1985; Bergé-Lefranc et al., 1985; Rabin et al., 1985) and contains an 8.5 kbp coding sequence divided into 48 exons, separated by introns varying in size up to 64 kbp (GenBank Accession Number: NT_008046) (Parma et al., 1987; Mendive et al., 1999, 2001; Moya et al., 2000).

The human TG mRNA is 8449–8468 bases long (Bergé-Lefranc et al., 1981; Brocas et al., 1982; Malthièry and Lissitzky, 1987; van de Graaf et al., 1997, 2001). The general organization of the sequence showed a 41-nucleotides 5′-untranslated segment, followed by a single open reading frame of 8307 bases and a 3′-untranslated segment ranging from 101 up to 120 bases. TG mRNA in human thyroid tissues is very heterogeneous due to 21 nucleotide polymorphisms (van de Graaf et al., 1997, 2001; Mendive et al., 1997; Hishinuma et al., 1999, 2006), 11 alternatively spliced transcripts (Mercken et al., 1989; Bertaux et al., 1991, 1995; Targovnik et al., 1992b; Mason et al., 1995) and 4 polyadenylation cleavage site variants (van de Graaf et al., 2001). The preprotein monomer is composed of a 19-amino acids signal peptide followed by a 2749 residues polypeptide (Malthièry and Lissitzky, 1987; van de Graaf et al., 2001). Analysis of the TG monomer primary sequence suggests that it is subdivided into regional structures (Fig. 3). 80% of the primary structure is characterized by the presence of three types of repetitive units: 11 Type 1, 3 Type 2 and 5 Type 3 (Fig. 3) (Malthièry and Lissitzky, 1987; van de Graaf et al., 2001). The remaining 20%, that constitutes the carboxy-terminal domain of the molecule, is not repetitive and exhibits 47% similarity to the whole length of acetylcholinesterase (Fig. 3) (Swillens et al., 1986; Park and Arvan, 2004).

TG and ACHE share certain common tertiary structural features. The six cysteine residues involved in ACHE intrachain disulfide bonds are conserved within TG (Swillens et al., 1986; Park and Arvan, 2004). The ACHE-homology domain is required for protein dimerization, which is essential for normal conformational maturation and intracellular transport of TG (Lee et al., 2009). Interestingly,

recent studies showed that attaching a signal peptide to the ACHE-like domain is itself sufficient for the efficient intracellular transport to extracellular space, indicating that ACHE-like domain may function as an intramolecular chaperone and as a molecular escort for Type 1, Type 2 and Type 3 TG domains (Lee et al., 2008).

After translation, intensive postranslational processes take place in the ER, Golgi aparatus, apical membrane and follicular lumen which include homodimers assembly, glycosylation, sialylation, sulfation, phosphorylation, iodination, multimerization and the formation of approximately 60 intrachain disulfide bonds per TG monomer. (Kim and Arvan, 1991; Sakurai et al., 1991; Yang et al., 1996; Berndorfer et al., 1996; Venot et al., 2002; Lee et al., 2009). Several ER chaperones, such as calnexin, Grp94, ERp72 and Bip interact with TG during its maturation and may serve to prevent export of improperly folded TG proteins (Kuznetsov et al., 1994; Kim and Arvan, 1995; Medeiros-Neto et al., 1996). This process is known as ER quality control.

Once TG has reached the follicular lumen, several tyrosines residues are iodinated and certain iodinated tyrosines are coupled to form T_3 and T_4. Four hormonogenic acceptor tyrosines have been identified and localized at positions 5 (exon 2), 1291 (exon 18), 2554 (exon 44) and 2747 (exon 48) in human TG (Fig. 3) (van de Graaf et al., 2001) and several tyrosines localized at positions 130 (exon 4), 847 (exon 10) and 1448 (exon 21) have been proposed as outer ring donor sites (Lamas et al., 1989). Tyrosine 5 is the most likely acceptor site for the donated iodotyrosyl from positions 130 (Palumbo et al., 1990; Dunn et al., 1998). The new secreted TG molecules would remain near the apical membrane of thyroid cells, where they would undergo hormone formation and be internalized and/or degraded rapidly. In contrast, the TG in the center of the follicle would be stored in the form of aggregates serving as an iodine and hormone reservoir. TG interacts with several proteins of the apical membrane in the exocytosis and endocytosis pathways of thyrocytes, such as apical membrane asialoglycoprotein receptor (ASGPR) (Miquelis et al., 1987; Montuori et al., 2000), megalin (Zheng et al., 1998; Marinò et al., 1999, 2000; Lisi et al., 2003), LDL receptor-associated protein (RAP) (Lisi et al., 2006), and protein disulfide isomerase (PDI) (Fig. 1) (Metzghrani et al., 1997, 2000). The ASGPR transports new synthesized TG to the follicular lumen. Highly iodinated TG is removed from the follicular lumen by internalization via pseudopod ingestion and micropinocytosis, followed by fusion of the endosome with a lysosome and its proteolytic cleavage. It has been reported that megalin, a member of the low-density lipoprotein receptor family, participates in the internalization of TG molecules as a high affinity receptor for TG (Marinò et al., 2000; Lisi et al., 2003). Megalin interacts with a heparin-binding region (SRRLKRP) in the carboxyl-terminal portion of rat TG (Marinò et al., 1999). However, this domain was not detected in human TG when we searched the complete TG protein for heparin-binding consensus sequences. Megalin contains a single transmembrane domain and is expressed in several polarized epithelial cells, and strictly on the apical surfaces of such cells. Megalin plays a role in intact TG transepithelial transport or transcytosis from apical to the basolateral surface of the thyrocyte (Fig. 1) (Marinò et al., 2000). RAP is an ER-resident protein that functions as a molecular chaperone for several members of the LDL receptor family, including megalin (Lisi et al., 2006). RAP is necessary for normal processing and subcellular distribution of megalin and interacts not only with megalin but also with TG, with relatively high affinity (Fig. 1). In the absence of RAP in thyrocytes, the folding of megalin is impaired and the receptor is retained within the ER (Lisi et al., 2006). In megalin-knockout mice the thyroid phenotype results in hypothyroidism and transfection of cells with a RAP devoid of the COOH-terminal ER retention signal results in impaired TG release with intracellularly retention or secretion into the bloodstream, suggesting that RAP may be involved in the TG biosynthetic or secretory pathway (Lisi et al., 2006).

It has been suggested that a quality control mechanism exists at the apical surface of the thyroid cell which prevents premature lysosomal transfer and degradation of immature TG (Metzghrani et al., 1997). The immature molecules are internalized and recycled through the trans-Golgi compartments (Fig. 1) (Miquelis et al., 1993). PDI is thought to be the candidate receptor that mediates the internalization (Metzghrani et al., 2000). The domain of TG responsible for the binding to membrane is located between exons 10 (Ser ^{789}) and 16 (Met ^{1173}). This region contains a stretch of 385 amino acid residues that includes the cysteine-rich motif Type 1–7, 1–8, 1–9, and 1–10 and two N-linked glycan moieties.

5. Thyroglobulin gene mutations and its clinical characteristics

The prevalence of patients with TG defects due to TG gene mutations is approximately 1 in 100,000 live births. Fifty mutations have been reported in the human TG gene: 23 missense mutations, 10 nonsense mutations, 5 single and 1 large nucleotide deletions, 1 single nucleotide insertion and 10 splice site mutations (Tables 1 and 2; Fig. 3). The clinical spectrum ranges from euthyroidism to severe hypothyroidism. Phenotypic variations among patients with the same mutations have been observed. The majority of patients have congenital goiter or goiter appearing shortly after birth.

The first family with a documented TG gene mutation were reported by Ieiri et al. (1991). In the index patient, a 33-year-old female from Japanese origin, a large asymmetrical and multinodular goiter was easily visible and palpable. The patient was the fifth of kindred of six, three of whom had a voluminous goiter. The parents were first cousins. Apart from the short stature, the physical examination was normal and the mental age was that of 13-year-old child. The hormonal status indicated severe hypothyroidism with low levels of TG. Molecular analysis showed that the defective synthesis of TG was due to the absence of exon 4 from the major TG transcript because of a cytosine to guanine homozygous transversion at position minus 3 in the acceptor splice site of intron 3 (g.IVS3-3C>G) (Tables 1 and 2) (Ieiri et al., 1991). Although removal of exon 4 does not modify the reading frame, the abnormal TG protein would lack 68 residues including the hormonogenic tyrosine at position 130.

A non-consanguineous Brazilian family with two affected siblings and a nephew presenting congenital goiter, severe hypothyroidism, and marked impairment of TG synthesis was extensively studied by Targovnik et al. (Targovnik et al., 1989, 1993, 1998; Gutnisky et al., 2004; Rivolta et al., 2005; Mendive et al., 2005; Caputo et al., 2007b). Both siblings had cretinous facies, an enlarged tongue, slow reflexes, short stature, coordination and speech difficulties and a multinodular goiter estimated to be about 6–8 times the normal size. Molecular studies indicated that the affected individuals are either compound heterozygous for c.886C>T/c.4588C>T (p.R277X/p.R1511) or c.886C>T/g.IVS34-1G>C mutations (Tables 1 and 2; Fig. 3) (Targovnik et al., 1993; Gutnisky et al., 2004). The same compound heterozygous mutations was also identified in two members of an Argentinian family (Tables 1 and 2) (Caputo et al., 2007b). Recently, Targovnik et al. (in press) reported the p.R1511X associated to the c.5386C>T [p.Q1777X] mutation in one member of a French family (Tables 1 and 2; Fig. 3).

The p.R277X mutation in exon 7 is the most frequently reported mutation in the TG gene (van de Graaf et al., 1999; Gutnisky et al., 2004; Rivolta et al., 2005; Caputo et al., 2007a, 2007b; Pardo et al., 2009; Machiavelli et al., 2010). The clinical spectrum of the homozygous patients with the p.R277X mutation ranges from moderate to severe goitrous hypothyroidism. The truncated form of TG

Table 1
Thyroglobulin gene mutations.

Exon/intron position	Nucleotide position	Amino acid position	References
Acceptor and donor splice site mutations			
Intron 3	g.IVS3+2T>G	Skipping of exon 3 and stop codon in the exon 4	Niu et al. (2009)
Intron 3	g.IVS3−3C<G	Skipping of exon 4	Ieiri et al. (1991)
Intron 5	g.IVS5+1G>A	Skipping of exon 5 and stop codon in the exon 6	Alzahrani et al. (2006)
Intron 10	g.IVS10−1G<A	Skipping of exon 11	Hishinuma et al. (2006)
Intron 24	g.IVS24+1G>C	Skipping of exon 24 and stop codon in the exon 26	Hishinuma et al. (2006)
Intron 30	g.IVS30+1G>T	Skipping of exon 30	Targovnik et al. (1995,2001); Pardo et al. (2008,2009)
Intron 30	g.IVS30+1G>A	Skipping of exon 30	Hishinuma et al. (2006)
Intron 34	g.IVS34−1G<C	Skipping of exon 35	Gutnisky et al. (2004)
Intron 45	g.IVS45+2T>A	Skipping of exon 45	Hishinuma et al. (2006)
Intron 46	g.IVS46−1G<A	Skipping of exon 46	Pardo et al. (2009)
Nonsense mutations			
Exon 7	c.886C>T	p.R277X	van de Graaf et al. (1999); Gutnisky et al. (2004); Rivolta et al. (2005); Caputo et al. (2007a, 2007b); Pardo et al. (2009); Machiavelli et al. (2010)
Exon 9	c.1351C>T	p.R432X	Niu et al. (2009)
Exon 9	c.2131C>T	p.Q692X	Hishinuma et al. (2006)
Exon 20	c.4310G>A	p.W1418X	Hishinuma et al. (2006)
Exon 22	c.4588C>T	p.R1511X skipping of exon 22	Targovnik et al. (1993, in press); Gutnisky et al. (2004); Mendive et al. (2005); Caputo et al. (2007b)
Exon 27	c.5350C>T	p.Q1765X	Niu et al. (2009)
Exon 27	c.5386C>T	p.Q1777X	Targovnik et al. (in press)
Exon 37	c.6481C>T	p.Q2142X	Pardo et al. (2009)
Exon 40	c.7006C>T	p.R2317X	Machiavelli et al. (2010)
Exon 46	c.7969C>T	p.Q2638X	Hishinuma et al. (2006)
Missense mutations			
Exon 2	c.113G>A	p.R19K	Kim et al. (2008)
Exon 5	c.548G>A	p.C164Y	Caputo et al. (2007a)
Exon 5	c.580T>G	p.C175G	Hishinuma et al. (2006)
Exon 8	c.986A>C	p.Q310P	Hishinuma et al. (2006)
Exon 10	c.2610G>T	p.Q851H	Corral et al. (1993); Pérez-Centeno et al. (1996)
Exon 11	c.2969G>A	p.S971I	Hishinuma et al. (2006)
Exon 12	c.3022C>T	p.R989C	Hishinuma et al. (2006)
Exon 12	c.3035C>T	p.P993L	Hishinuma et al. (2006)
Exon 14	c.3229T>C	p.C1058R	Hishinuma et al. (2005, 2006)
Exon 17	c.3790T>C	p.C1245R	Hishinuma et al. (1999, 2005, 2006); Baryshev et al. (2004); Kanou et al. (2007)
Exon 24	c.4820G>T	p.C1588F	Hishinuma et al. (2006)
Exon 21	c.4397G>A	p.S1447N	Hishinuma et al. (2006)
Exon 31	c.5690G>A	p.C1878Y	Kitanaka et al. (2006); Hishinuma et al. (2006)
Exon 31	c.5791A>G	p.I1912V	Hishinuma et al. (2006)
Exon 33	c.5986T>A	p.C1977S	Hishinuma et al. (1999, 2005, 2006); Baryshev et al. (2004)
Exon 33	c.6017G>A	p.C1987Y	Hishinuma et al. (2006)
Exon 37	c.6461G>A	p.C2135Y	Hishinuma et al. (2006)
Exon 38	c.6701C>A	p.A2215D	Caputo et al. (2007a); Pardo et al. (2008, 2009); Machiavelli et al. (2010)
Exon 38	c.6725G>A	p.R2223H	Caron et al. (2003); Machiavelli et al. (2010)
Exon 40	c.6956G>A	p.G2300D	Hishinuma et al. (2006)
Exon 40	c.7007G>A	p.R2317Q	Kitanaka et al. (2006); Hishinuma et al. (2006)
Exon 41	c.7121G>T	p.G2355V	Hishinuma et al. (2006)
Exon 41	c.7123G>A	p.G2356R	Hishinuma et al. (2005, 2006); Kanou et al. (2007)
Single nucleotide insertion			
Exon 7	c.759–760insA	p.L234fsX237	Caputo et al. (2007a)
Single nucleotide deletions			
Exon 9	c.1143delC	p.G362fsX382	Caron et al. (2003)
Exon 9	c.1348delT	p.S431fsX459	Niu et al. (2009)
Exon 9	c.1712delT	p.L552fsX576	Niu et al. (2009)
Exon 22	c.4537delG	p.D1494fsX1547	Hishinuma et al. (2006)
Exon 33	c.6047delA	p.Q1997fsX1998	Niu et al. (2009)
Large nucleotide deletion			
Deletion in the 5′ region of the TG gene that involves promoter region and 11 first exons			González-Sarmiento et al. (2001)

The nucleotide position is designated according to TG mRNA reference sequences (GenBank Accesion Number: NM_003235). The A of the ATG of the initiator methionine codon is denoted nucleotide + 1. The amino acid positions are numbered after subtracting the 19-amino acid signal peptide. Intronic nucleotides located upstream of the exon have negative numbering, while those located downstream have positive numbering. Frame shifting mutations are designated by "fs" after a description of the first amino acid affected by the change (insertion or deletion) and followed by "X", that indicates at which codon position the new reading frame ends in a stop.

Table 2
Thyroglobulin mutations list according to homozygous or compound heterozygous state and country of origin.

Country	Nucleotide position	Amino acid position	References
Argentina			
1	c.548G>A/c.759–760insA	p.C164Y/p.L234fsX237	Caputo et al. (2007a)
2	c.886C>T/c.886C>T	p.R277X/p.R277X	Rivolta et al. (2005); Caputo et al. (2007a)
3	c.886C>T/c.6701C>A	p.R277X/p.A2215D	Caputo et al. (2007a)
4	c.886C>T/c.4588C>T	p.R277X/p.R1511X	Caputo et al. (2007b)
5	c.886C>T/not identified	p.R277X/not identified	Machiavelli et al. (2010)
6	c.6701C>A/c.6701C>A	p.A2215D/p.A2215D	Machiavelli et al. (2010)
7	c.6725G>A/c.7006C>T	p.R2223H/p.R2317X	Machiavelli et al. (2010)
Brazil			
1	c.886C>T/c.886C>T	p.R277X/p.R277X	van de Graaf et al. (1999); Pardo et al. (2009)
2	c.886C>T/c.4588C>T	p.R277X/p.R1511X	Gutnisky et al. (2004); Caputo et al. (2007b)
3	c.886C>T/c.6701C>A	p.R277X/p.A2215D	Pardo et al. (2009)
4	c.886C>T/g.IVS34-1G>C	p.R277X/skipping exon 35	Gutnisky et al. (2004)
5	c.886C>T/g.IVS46-1G>A	p.R277X/skipping exon 47	Pardo et al. (2009)
6	g.IVS30+1G>T/g.IVS30+1G>T	Skipping exon 30/skipping exon 30	Targovnik et al. (2001); Pardo et al. (2008)
7	g.IVS30+1G>T/c.6701C>A	Skipping exon 30/p.A2215D	Pardo et al. (2009
8	c.6481C>T/c.6481C>T	p.Q2142X/p.Q2142X	Pardo et al. (2009)
9	c.6701C>A/c.6701C>A	p.A2215D/p.A2215D	Pardo et al. (2009)
Spain			
1	c.2610GT/not identified	p.P.Q851H/not identified	Corral et al. (1993); Pérez-Centeno et al. (1996)
2 Monoallelic deletion in the 5′ region of the TG gene that involves promoter region and 11 first exons			González-Sarmiento et al. (2001)
France			
1	c.4588C>T/c.5386C>T	p.R1511X/p.Q1777X	Targovnik et al. (in press)
2	c.1143delC/c.6725G>A	p.G362fsX382/p.R2223H	Caron et al. (2003)
Japan			
1	g.IVS3-3C>G/g.IVS3-3G>C	Skipping exon 4/skipping exon 4	Ieiri et al. (1991)
2	c.580T>G/c.580T>G	p.C175G/p.C175G	Hishinuma et al. (2006)
3	c.986A>C/g.IVS30+1G>A	p.Q310P/skipping exon 30	Hishinuma et al. (2006)
4	c.986A>C/c.6461G>A	p.Q310P/p.C2135Y	Hishinuma et al. (2006)
5	c.2969G>A/c.3035C>T	p.S971I/p.P993L	Hishinuma et al. (2006)
6	g.IVS10-1G>A/g.IVS10-1G>A	Skipping exon 11/skipping exon 11	Hishinuma et al. (2006)
7	c.3229T>C/c.3229T>C	p.C1058R/p.C1058R	Hishinuma et al. (2005, 2006)
8	c.3790T>C/c.3790T>C	p.C1245R/p.C1245R	Hishinuma et al. (1999, 2005, 2006)
9	c.3790T>C/c.2131C>T	p.C1245R/p.Q692X	Hishinuma et al. (2006)
10	c.3790T>C/c.4537delG	p.C1245R/p.D1494fsX1547X	Hishinuma et al. (2006)
11	c.3790T>C/g.IVS24+1G>C	p.C1245R/skipping exon 24	Hishinuma et al. (2006)
12	c.3790T>C/c.7123G>A	p.C1245R/p.G2356R	Hishinuma et al. (2005, 2006); Kanou et al. (2007)
13	c.3790T>C/c.7969C>T	p.C1245R/p.Q2638X	Hishinuma et al. (2006)
14	c.4397G>A/c.3022C>T	p.S1447N/p.R989C	Hishinuma et al. (2006)
15	c.4397G>A/c.7123G>A	p.S1447N/p.G2356R	Hishinuma et al. (2006)
16	c.4820G>T/c.4820G>T	p.C1588F/p.C1588F	Hishinuma et al. (2006)
17	c.4820G>T/c.4310G>A	p.C1588F/p.W1418X	Hishinuma et al. (2006)
18	c.5690G>A/c.7007G>A	p.C1878Y/p.R2317Q	Kitanaka et al. (2006); Hishinuma et al. (2006)
19	c.5791A>G/c.6017G>A	p.I1912V/p.C1987Y	Hishinuma et al. (2006)
20	c.5986T>A/c.5986T>A	p.C1977S/p.C1977S	Hishinuma et al. (1999, 2005, 2006)
21	c.5986T>A/c.4820G>A	p.C1977S/p.C1588F	Hishinuma et al. (2006)
22	c.6956G>A/c.6956G>A	p.G2300D/p.G2300D	Hishinuma et al. (2006)
23	c.7121G>T/g.IVS45+2T>A	p.G2355V/skipping exon 45	Hishinuma et al. (2006)
Taiwan			
1	c.1348delT/c.1351C>T	p.S431fsX459/p.R432X	Niu et al. (2009)
2	c.1351C>T/c.1712delT	p.R432X/p.L552fsX576	Niu et al. (2009)
3	g.IVS3+2T>G/c.5350C>T	Skipping exon 3/p.Q1765X	Niu et al. (2009)
4	g.IVS3+2T>G/c.6047delA	Skipping exon 3/p.Q1997fsX1998	Niu et al. (2009)
5	c.1348delT/c.1348delT	p.S431fsX459/p.S431fsX459	Niu et al. (2009)
Saudi Arabia			
1	g.IVS5+1G>A/g.IVS5+1G>A	Skipping exon 5/skipping exon 5	Alzahrani et al. (2006)

The nucleotide position is designated according to TG mRNA reference sequences (GenBank Accession Number: NM_003235). The A of the ATG of the initiator methionine codon is denoted nucleotide + 1. The amino acid positions are numbered after subtracting the 19-amino acid signal peptide. Intronic nucleotides located upstream of the exon have negative numbering, while those located downstream have positive numbering. Frame shifting mutations are designated by "fs" after a description of the first amino acid affected by the change (insertion or deletion) and followed by "X", that indicates at which codon position the new reading frame ends in a stop.

still harbors both the acceptor tyrosine 5 and the donor tyrosine 130. However, the premature stop codon eliminates the carboxy-terminal hormonogenic domain, resulting in the loss of thyroid hormone formation. *In vitro* expression of the truncated p.R277X TG cDNA showed that the mutated TG protein can be glycosylated, indicating their possible export to apical surface of thyrocytes (van de Graaf et al., 1999). Recently, it has been reported that the introduction of a stop codon in place of mouse TG cysteine 175 still allows the secretion of a truncated protein (Kim et al., 2008). This observation strongly suggests that the secretory pathway continues to work despite the complete deletion of ACHE-like region, as is observed in the p.R277X peptide. The RT-PCR analysis did not find evidence for exon skipping that could restore the normal reading frame disrupted by the nonsense mutation (van de Graaf et al., 1999). The explanation for this observation may be that the p.R277X is a terminal codon in exon 7 and not disrupt any exonic splicing

enhancers (ESE) elements implicated in exon definition and inclusion (Machiavelli et al., 2010). ESEs are sequence tracts present in constitutive and alternative exons that are required for correct exon definition and splicing.

The comparison of the TG polymorphic markers identified in a homozygous Argentinian patient with a member of a Brazilian family, who also carried the p.R277X as a compound heterozygous mutations (Rivolta et al., 2005), reveled that the two affected individuals do not share a common TG allele, supporting that a mutational hot spot mechanism is responsible of the p.R277X mutation. However, comparative analysis between the haplotypes segregating with the mutation p.R277X from two Argentinian families suggests the possibility that this mutation was derived from a common ancestral chromosome (Caputo et al., 2007b).

In contrast to p.R277X, p.R1511X mutation in exon 22 is removed from the transcripts by exon skipping (Targovnik et al., 1993; Gutnisky et al., 2004; Mendive et al., 2005; Caputo et al., 2007b) and there is a preferential accumulation in the goiter of a TG mRNA lacking exon 22. Skipping of mutated exon 22 in the pre-mRNA restores the reading frame allowing translation to reach the normal stop codon. This alternative splicing is also present in mRNA from normal thyroid tissue, but it represents a minor fraction of the total TG transcripts (Targovnik et al., 1992b). The excision of exon 22 in the TG mRNA results in an in-frame deletion of 57 amino acid residues, which is localized in the TG Type-1 repeat motif. The p.R1511X mutation, together with the p.R687X (c.2146C>T) mutation documented in the exon 9 of the Afrikander cattle TG gene also illustrate the phenomenon of nonsense-mediated altered splicing, in which an exon harboring a premature stop codon is removed from the mature transcript (Ricketts et al., 1987; Mendive et al., 2005). The functional consequences of the deletion of exon 22 could be structural changes in the protein with retention of the molecule within the cell. Alternatively, it is possible that the elimination of exons containing repeat motifs by alternative splicing results in an altered ability to transfer an iodophenoxyl group from the donor site to the acceptor iodotyrosine in the coupling machinery (Gutnisky et al., 2004). Cysteine residues are thought to play an important role in the tertiary structure of TG, 5 of which are localized in exon 22 (Targovnik et al., 1992b, 1993; Gutnisky et al., 2004). Different haplotypes segregated with the p.R1511X mutation demonstrating the absence of a founder effect for these mutations between Argentinian and Brazilian populations (Caputo et al., 2007b).

The c.886C>T and c.4588C>T nonsense mutations occur in a CpG dinucleotide sequence and could be caused by deamination of a methylated cytosine resulting in a thymine. The CGA arginine codon is considered a hot spot for mutations in mammalian DNA.

Another homozygous aberrant splicing due to a guanine to thymine transversion at position +1 in the donor splice site of intron 30 (g.IVS30+1G>T) was identified in two members of a Brazilian family with a history of congenital goiter (Tables 1 and 2) (Targovnik et al., 1995; Targovnik et al., 2001). The family pedigree confirmed the presence of a consanguineous marriage and the same clinical and biological abnormalities in four siblings. Two presumably affected hypothyroid children died during the neonatal period and at the age of 7 months. Both affected patients studied had goiter and severe hypothyroidism being homozygous for the mutation (Targovnik et al., 1995). The thyroid scan was compatible with multinodular goiter and ultrasonography of the thyroid indicated marked heterogeneity of the echographic responses with large hyperechoic nodules. Serum TG levels were below the limit of detection. The elimination of 138 nucleotides corresponding to exon 30 does not affect the reading frame of the resulting mRNA and is potentially fully translatable into a TG polypeptide chain that is shortened by 46 residues (Targovnik et al., 1995, 2001). A glycine residue is maintained by the junction between the proximal G from glycine 1831 and the distal GT from arginine 1877. The functional consequences of the deletion are related to structural changes in the protein molecule that either could modify the normal routing of the translation product through the membrane system of the cell or could impair the coupling reaction (Medeiros-Neto et al., 1996). The deletion causes the loss of 1 putative N-linked glycosylation site.

Hishinuma et al. (1999) suggest that missense mutations that replace cysteine cause an abnormal three-dimensional structure of TG and consequently, its defective intracellular transport. Two new missense mutations in exons 17 and 33 were identified in two unrelated patients with congenital goiter and two siblings with the variant type of adenomatous goiter (Hishinuma et al., 1999). The thyroid function of these four patients ranged from euthyroidism to mild hypothyroidism. The cytosine to thymine homozygous transition at position 3790 (c.3790C>T) which caused an amino acid substitution from cysteine to arginine at codon 1245 (p.C1245R) was detected in the patients with congenital goiter (Tables 1 and 2; Fig. 3). Whereas, the thymine to adenine homozygous transversion at position 5986 (c.5986T>A) which caused an amino acid substitution from cysteine to serine at codon 1977 (p.C1977S) was detected in the siblings with the variant type of adenomatous goiter (Tables 1 and 2; Fig. 3). Baryshev et al. (2004) demonstrated that p.C1245R and p.C1977S mutations in the human, as well as p.G2300R mutation in the rdw rat induce the unfolded protein response (UPR). UPR is an adaptive cellular reaction that regulates the protein folding capacity of the ER which is perturbed by the excessive accumulation of the mutant secretory proteins. The UPR includes a transcriptional induction of molecular chaperones, via enhanced splicing of X-box binding protein (XBP1) or processing of activating transcription factor 6 (ATF6) and general translational attenuation by PERKR-like ER kinase. In normal conditions, these sensors are silenced by interaction with a major ER chaperone, BiP. The processing of ATF6 was observed in both human and rat tissues with TG mutations, whereas XBP1 splicing was detected only in the p.C1245R mutant (Baryshev et al., 2004).

The first report of a mutation in the ACHE-homology domain of TG in humans was observed in a French family with two affected siblings with congenital goitrous hypothyroidism (Caron et al., 2003). The mutation located in the ACHE-homology domain is associated with a mutation in the large exon 9, showing that the patient had a new compound heterozygous mutations (Tables 1 and 2; Fig. 3). A fetal goiter was diagnosed in both patients by ultrasound at the sixth month of gestation. Percutaneous umbilical vein blood sampling was carried out under ultrasound guidance showing severe fetal hypothyroidism. Biochemical evaluation of both neonates revealed low serum TG concentration, high serum TSH and low free T4. The paternal mutation consists of a cytosine deletion at nucleotide position 1143 in exon 9 (c.1143delC), resulting in a frameshift at amino acid 362 which generates a stop codon at position 382 in the same exon (p.362fsX382) (Caron et al., 2003) The maternal mutation is a guanine to adenine substitution at position 6725 in exon 38 (c.6725G>A), creating the p.R2223H missense mutation in the ACHE-homology domain of TG (Caron et al., 2003). The wild-type arginine residue at position 2223 is strictly conserved in all species for which suitable TG and ACHE sequences have been reported (Caron et al., 2003). Computer analysis of the protein's secondary structure showed that the p.R2223H mutation causes an extended stretch of the helix structure. Consequently, the arginine residue in this position plays a critical structural role in the TG protein. The functional consequences of this mutation could be the retention of the TG protein inside the ER due to structural alterations, as already observed in another missense mutations localized to the ACHE-homology domain in the *cog*/*cog* congenital goiter mouse (c.6848T>C [p.L2263P]) and the *rdw*/*rdw* rat (c.6958G>C [p.G2300R]) (Kim et al., 1998, 2000; Hishinuma et

al., 2000). In *cog/cog* mice full-length TG is synthesized, but its folding is defective, consequently, the proteins never arrive at the Golgi complex (Kim et al., 1998). The correction of this missense mutation restores the normal TG secretion (Kim et al., 1998). The *rdw/rdw* rat is a hereditary hypothyroid variant derived from the Wistar–Imamichi strain (Hishinuma et al., 2000; Kim et al., 2000). In contrast to human and *cog/cog* mouse model, the *rdw/rdw* rat presents a hypoplastic thyroid gland that was smaller that the normal control, despite the elevated circulating levels of TSH and the reduced level of T_3 and T_4. As in cog/cog mice, the rdw TG was retained inside the ER in cells (Hishinuma et al., 2000; Kim et al., 2000).

Interestingly, Hishinuma et al. (2005) reported patients with the TG gene mutations associated with thyroid cancer development. These alterations are identified in papillary and follicular carcinoma. Sequence analysis revealed two previously described TG mutations (p.C1245R and p.C1977S) and two novel mutations (c.3229T>C [p.C1058R] and c.7123G>A or [p.G2356R]) as homozygous (p.C1058R, p.C1245R and p.C1977S) or compound heterozygous (p.C1245R/p.G2356R) state (Tables 1 and 2; Fig. 3). Subsequently, genomic sequencing of exon 15 of the BRAF gene, in cancerous tissue, revealed two heterozygous activating mutations (p.V599E and p.K600E) in two of these patients Hishinuma et al. (2005).

Alzahrani et al. (2006) identified a guanine to adenine homozygous transition at position +1 of the splice donor site of intron 5 (g.IVS5+1G>A) in TG gene in two brothers with recurrent huge goiters and severe hypothyroidism (Tables 1 and 2) (Alzahrani et al., 2006). One of them developed a widely metastatic follicular thyroid carcinoma. Both parents are first-degree relatives and were heterozygous for the same mutation, and no mutation was found in their unaffected brother. Both siblings had difficulty in their school performance. Neck ultrasound revealed multiple thyroid nodules. Serum TG was undetectable. The fusion of exons 4 and 6 leads to a reading frame shift at position arginine 141 and a premature stop codon at position 147, in the same exon 6 (Alzahrani et al., 2006). This truncated form of TG harbors the acceptor Tyr 5 and the donor Tyr 130 residues as well as two putative N-linked glycosylation sites.

Some cases of endemic and euthyroid nonendemic simple goiter are associated with a mutation within exon 10 of the TG gene. Sequencing analysis revealed a mutation at position 2610, which implies a G to T substitution (c.2610G>T) (Tables 1 and 2). This single base change results in a glutamine to histidine substitution (p.Q851H) (Corral et al., 1993; Pérez-Centeno et al., 1996). Monoallelic deletion in the 5′ region of the TG gene was observed also associated to nonendemic simple goiter (González-Sarmiento et al., 2001). Approximately, the deletion involves the promoter region and the 11 first exons of this gene (Tables 1 and 2).

In the last years TG gene mutation was extensively screened in Japanese, Argentinian, Brazilian and Taiwanese patients with TG defects (Tables 1 and 2; Fig. 3).

Hishinuma et al. (2006) have reported 26 different inactivating mutations in the TG gene in 52 congenital goiters euthyroid or mildly hypothyroid from 41 families, within the japanese population, 22 are novel mutations: 14 missense mutations (c.580T>G [p.C175G], c.986A>C [p.Q310P], c.2969G>A [p.S971I], c.3022C>T [p.R989C], c.3035C>T [p.P993L], c.4397G>A [p.S1447N], c.4820G>T [p.C1588F], c.5690G>A [p.C1878Y], c.5791A>G [p.I1912V], c.6017G>A [p.C1987Y], c.6461G>A [p.C2135Y], c.6956G>A [p.G2300D], c.7007G>A [p.R2317Q] and c.7121G>T [p.G2355V]), 4 splice mutations (g.IVS10-1G>A, g.IVS24+1G>C, g.IVS30 + 1G>A and g.IVS45 + 2T>A), 3 nonsense mutations (c.2131C>T [p.Q692X], c.4310G>A [p.W1418X] and c.7969C>T [p.Q2638X]) and 1 single nucleotide deletion (c.4537delG [p.D1494fsX1547]) (Tables 1 and 2; Fig. 3). The remaining four previously reported mutations are p.C1058R, p.C1245R, p.C1977S and p.G2356R (Hishinuma et al., 1999, 2005; Baryshev et al., 2004). Thirty-five patients were homozygous, whereas 17 patients were compound heterozygous. The patients harboring the frequent mutations p.C1058R and p.C1977S show the same combinations of the single nucleotide polymorphisms in the coding region of the TG gene. Consequently, this finding suggests that the occurrence of these mutations is due to a founder effect. The patient with the compound heterozygous for p.C1878Y/p.R2317Q mutations has a serum T_3 concentration disproportionately higher compared with T_4 (Kitanaka et al., 2006). In this respect Kanou et al. (2007) identified that the compound heterozygous for p.C1245R/p.G2356R mutations is associated with increased thyroidal Type-2 iodothyronine deiodinase activity and propose that this increase is the possible mechanism responsible for the higher plasma T_3 concentration.

Screening by direct sequencing analysis of the TG gene from eight patients from six non-consanguineous Argentinian families with congenital hypothyroidism revealed four novel mutations (c.548G>A [p.C164Y], c.759–760insA [p.L234fsX237], c.6701C>A [p.A2215D] and c.7006C>T [p.R2317X]) and three previously reported mutation (p.R277X, p.R1511X and p.R2223H), constituting four compound heterozygous for p.C164Y/p.L234fsX237, p.R277X/p.A2215D, p.R277X/p.R1511X and p.R2223H/p.R2317X mutations and two homozygous for p.R277X, and p.A2215D mutations (Tables 1 and 2; Fig. 3) (Caputo et al., 2007a; Machiavelli et al., 2010). Two siblings had the homozygous p.A2215D mutation and another two had the compound heterozygous for p.R277X/p.R1511X mutations. In addition, three patients from two unrelated families that were also studied by congenital hypothyroidism had a single p.R277X mutated allele (Machiavelli et al., 2010). It is unclear whether the phenotype is caused by a monoallelic defect since deletion of a single exon, or mutations in distant regulatory regions of the TG gene or in remote intronic regions cannot be excluded.

The clinical spectrum of the resulting phenotypes of all Argentinian patients ranges from mild to severe goitrous hypothyroidism. Six patients were detected by neonatal screening and treated in the first days of life. The remaining 5 were detected between 1.3 and 4 years of age and, in spite of late treatment have normal development or mild retardation (Caputo et al., 2007a; Machiavelli et al., 2010).

Recently, genetic screening of 17 patients from 11 unrelated Brazilian families with congenital hypothyroidism due to TG deficiency was reported by Pardo et al. (2008, 2009). All patients had an absent rise of serum TG after stimulation with recombinant human TSH. Sequence analysis revealed three previously described mutations (p.R227X, g.IVS30+1G>T and p.A2215D) and two novel mutations (c.6481C>T [p.Q2142X] and g.IVS46−1G>A) (Tables 1 and 2; Fig. 3) (Pardo et al., 2008, 2009). The p.A2215D mutation was found on both alleles in six patients. A single p.A2215D mutation was identified in other three patients of which two siblings harbored also the known mutation g.IVS30+1G>T and the remaining unrelated patient the previously described p.R277X mutation, constituting two compound heterozygous for p.R277X/p.A2215D and g.IVS30+1G>T/p.A2215D mutations. The p.R277X and g.IVS30+1G>T mutations were found in homozygosity in 3 and 2 patients, respectively, whereas the new p.Q2142X mutation was found in both alleles in two siblings. The other novel mutation identified g.IVS46−1G>A was found in heterozygosity in one patient who also harbored the p.R277X mutation. It is interesting to note that the homozygous patients for the p.A2215D had euthyroidism or mild hypothyroidism, in contrast, the homozygous patients with the p.R277X or g.IVS30+1G>T or p.Q2142X mutations had severe hypothyroidism (Pardo et al., 2009). However, functional analysis suggests that the mutation p.A2215D results in retention of the

molecule within the RE. A possible explanation to this disagreement is that the presence of sufficient iodine supply in some patients with homozygote p.A2215D mutation is able to compensate the dishormonogenesis. An alternative explanation is that some p.A2215D TG molecules may escape from the RE (Pardo et al., 2009).

More recently, six new mutations of the TG gene were reported in 7 patients from 6 Taiwanese families with severe hypothyroidism, including 1 aberrat splicing (g.IVS3+2T>G), 2 nonsense mutations (c.1351C>T [p.R432X] and c.5350C>T [p.Q1765X]) and 3 single nucleotide deletion (c.1348delT [p.S431fsX459], c.1712delT [p.L552fsX576] and c.6047delA [p.Q1997fsX1998]) (Tables 1 and 2; Fig. 3) (Niu et al., 2009). Constituting four compound heterozygous for p.R432X/p.L552fsX576, p.S431fsX459/p.R432X, g.IVS3+2T>G/p.Q1765X and g.IVS3+2T>G/p.Q1997fsX1998 mutations and one homozygous for p.S431fsX459 mutation. The g.IVS3+2T>G mutation causes the skipping of exon 3, resulting in a frameshift which generates a stop codon after 17 altered amino acid residues. The patient homozygous for p.S431fsX459 or harbored g.IVS3+2T>G/p.Q1765X or g.IVS3+2T>G/p.Q1997fsX1998 mutations do not have clinical goiter (Niu et al., 2009). Whereas, the patients carrying the compound heterozygous for p.R432X/p.L552fsX576 mutations had goiter detectable only by palpation. The absence of an evidenced goiter can be presumed to the fact that the patients were detected and treated in the neonatal period as the result of confirmation of an abnormal neonatal screening. Haplotype analysis revealed that the p.S431fsX459 mutation is due to a founder effect, whereas p.R432X seems caused by independently recurrent de novo mutations (Niu et al., 2009).

6. Conclusions and perspectives

The identification of 50 mutations linked with goiter and hypothyroidism or euthyroidism confirms the allelic heterogeneity of the TG gene mutations. TG gene defects are inherited in an autosomal recessive manner and affected individuals are either homozygous or compound heterozygous for mutations in the TG gene. Mutations described have been classified as missense and nonsense mutations, frameshift mutations by single nucleotide deletion or insertion, large 5′ gene deletion and splicing mutations in the exonic or intronic consensus sites. Several TG mutants can be blocked in his ER export and then are routed for proteasomal degradation. However, truncated TG proteins before the ACHE-homology C-terminal region domain can be secreted and are sufficient for partial thyroid hormone synthesis. The thyroid gland develops hypertrophy and hyperplasia by the proliferative effect as a consequence of the chronic elevation of TSH.

Regardless of these advances little is known about the structure–function relationship of the TG because of our lack of knowledge about the three-dimensional structure of this protein. Unfortunately, there are no X-ray crystallographic data of any TG regions. The analysis of the primary structure of the TG protein shows eight domains: three families of cysteine-rich repetitive units, one ACHE-homology C-terminal region and four hormonogenic sites. However, many identified TG domains remain functionally uncharacterized and the further investigations combining genotyping analysis, patients' phenotype, expression studies and computational modelling are necessary for elucidating the function/properties of these domains. The continued study of TG mutations may be helpful for understanding the pathophysiology of hereditary hormonogenic diseases.

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