Available online at www.sciencedirect.com
ScienceDirect
Clinica Chimica Acta 374 (2006) 8–24
www.elsevier.com/locate/clinchim

Invited critical review

Molecular advances in thyroglobulin disorders

Carina M. Rivolta, Héctor M. Targovnik *

Laboratorio de Biología Molecular, Cátedra de Genética y Biología Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 - Buenos Aires, Argentina

Received 27 March 2006; received in revised form 29 May 2006; accepted 30 May 2006
Available online 7 June 2006

Abstract

Synthesis of tri-iodothyronine (T_3) and thyroxine (T_4) follows a metabolic pathway that depends on the integrity of the thyroglobulin structure. This large glycoprotein is a homodimer of 660 kDa synthesized and secreted by the thyroid cells into the lumen of thyroid follicle. In humans it is coded by a single copy gene, 270 kb long, that maps on chromosome 8q24 and contains an 8.5 kb coding sequence divided into 48 exons. The preprotein monomer is composed of a 19-amino acid signal peptide followed by a 2749-amino acid polypeptide.

In the last decade, several mutations in the thyroglobulin gene were reported. In animals, four of them have been observed in Afrikander cattle (p.R697X), Dutch goats (p.Y296X), cog/cog mouse (p.L2263P) and rdw rats (p.G2300R). Mutations in the human thyroglobulin gene are associated with congenital goiter or endemic and nonendemic simple goiter. Thirty-five inactivating mutations have been identified and characterized in the human thyroglobulin gene: 20 missense mutations (p.C175G, p.Q310P, p.Q851H, p.S971I, p.R989C, p.P993L, p.C1058R, p.C1245R, p.S1447N, p.C1588F, p.C1878Y, p.I1912V, p.C1977S, p.C1987Y, p.C2135Y, p.R2223H, p.G2300D, p.R2317Q, p.G2355V, p.G2356R), 8 splice site mutations (g.IVS3−3C>G, g.IVS5+1G>A, g.IVS10−1G>A, g.IVS24+1G>C, g.IVS30+1G>T, g.IVS30+1G>A, g.IVS34−1G>C, g.IVS45+2T>A) 5 nonsense mutations (p.R277X, p.Q692X, p.W1418X, p.R1511X, p.Q2638X) and 2 single nucleotide deletions (p.G362fsX382, p.D1494fsX1547).

The thyroglobulin gene has been also identified as the major susceptibility gene for familial autoimmune thyroid diseases (AITD) by linkage analysis using highly informative polymorphic markers. In conclusion the identification of mutations in the thyrogobulin gene has provided important insights into structure–function relationships.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Thyroglobulin gene; Thyroglobulin defect; Congenital goiter; Hypothyroidism; Familial euthyroid goiter

Contents

1. Introduction . . . 9
2. The nucleotide and amino acid nomenclatures . . . 10
3. The thyroglobulin gene: structure, expression and regulation . . . 10
4. Thyroglobulin gene molecular markers . . . 13
5. Human thyroglobulin gene mutations . . . 13
6. Animal thyroglobulin gene mutations . . . 16
7. Thyroglobulin gene mutations in patients with nonendemic and endemic goiter . . . 18
8. Risk of non-medullary thyroid cancer associated to the p.R2511Q thyroglobulin gene polymorphism . . . 18
9. Thyroglobulin gene is an important susceptibility gene for autoimmune thyroid disease . . . 18
10. Conclusions and perspectives . . . 18
Acknowledgements. . . . 19
References . . . 19

* Corresponding author. Tel.: +54 11 4964 8296; fax: +54 11 4508 3645.
E-mail address: htargovn@huemul.ffyb.uba.ar (H.M. Targovnik).

0009-8981/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2006.05.043

1. Introduction

Normal biosynthesis of thyroid hormones requires the integrity of a complex protein system and several sequential steps [1–5] . The most abundant expressed protein in the thyroid gland is thyroglobulin (Tg), which functions as the matrix for thyroid hormone synthesis and in the storage of the inactive form of thyroid hormone and of the iodine. Iodine enters the thyroid follicular cell as inorganic iodide and is transformed in organic form by the iodination of specific iodotyrosyl residues of Tg [5]. The iodide transport consists of two steps and involves 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 [6]. On the other hand, the pendrin, located in the apical membrane, is responsible for the iodide transport from epithelial cell to follicular lumen [7,8]. Rodriguez et al. identified a new putative iodide transport in the apical membrane, named hAIT for human Apical Iodide Transporter [9].

The step preliminary to thyroid hormone formation is the oxidation of iodide, then, the attachment of iodine to tyrosyl residues in Tg is produced to generate MIT and DIT [5]. The final step is the coupling of two iodotyrosyl residues to form iodothyronine, two DIT form T_4, one DIT and one MIT form T_3 (Fig. 1). TPO is the key enzyme in the thyroid hormones formation; it catalyzes both the iodination and coupling of hormonogenic tyrosyl residues of Tg with an absolute requirement of hydrogen peroxide, which acts as an electron acceptor [10–12]. H_2O_2 is generated on the apical plasma membrane of the thyroid follicular cell by a metabolic pathway, involving two members of the NADPH oxidase family (Duox1 and Duox2) [13,14]. Recently, a thiredoxin-related protein named EFP1 (EF-hand binding protein 1) was identified as a novel partner in the assembly of the multiprotein complex constituting the thyroid H_2O_2 generating system [15]. Afterwards, the hormones are released from Tg by proteolysis (Fig. 1). The action of thyroid hormones is mediated by T_3, by binding to the nuclear receptor [2,5]. Thyroid hormone receptors regulate the transcription of target genes by binding to

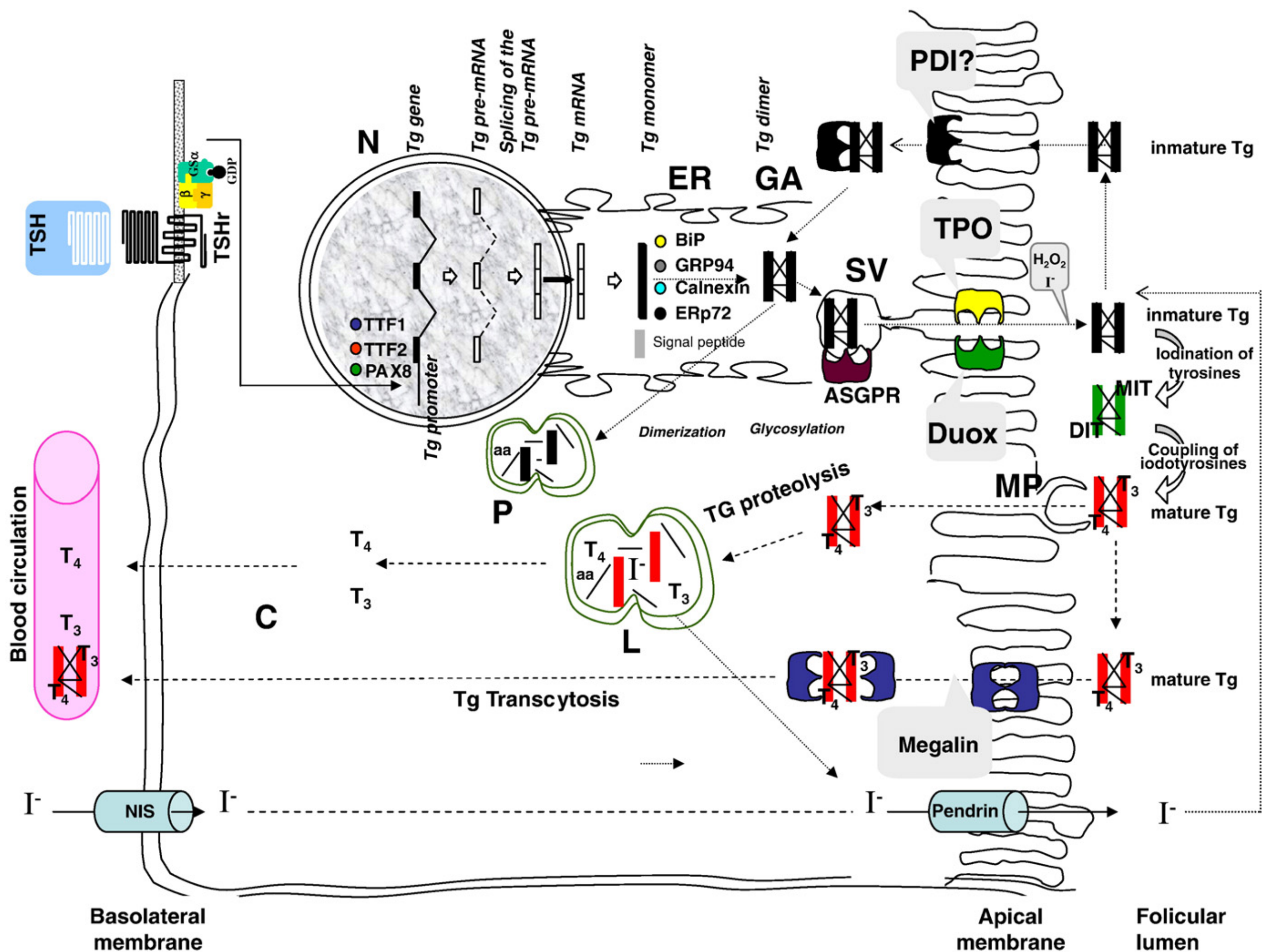

Fig. 1. Schematic representation of the maturation, internalization and transepithelial transport pathways of the thyroglobulin protein within the thyroid cells. The relevant processes in the different organelles and the thyroid-specific proteins are shown. N, nucleous; ER, endoplasmic reticulum; GA, Golgi apparatus; P, peroxisome; L, lysosome; SV, secretory vesicles; MP micropinocytosis; 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; NIS, sodium iodide symporter; Duox, dual oxidase; PDI, protein disulfide isomerase; ASGPR, asialoglycoprotein receptor.

thyroid hormone response elements (TREs) in their promoter regions. Thyroid hormone receptors bind to TREs as homodimers or heterodimers with retinoid X receptors.

The general organization of the Tg gene and its mRNA has been studied in detail by several groups [16–45]. Deeper function–structure relation features remain unresolved because of our lack of knowledge about the three-dimensional structure of the protein.

Inactivating mutations of the Tg gene have been identified in humans in the last decade, resulting in structural defects and endoplasmic reticulum retention of Tg proteins, and have been linked to subsequent thyroid hormone-impaired and primary congenital goiter with hypothyroidism or euthyroidism [46–65]. Mutations in Tg gene also have been reported associated with endemic [66] and nonendemic goiter [67,68]. Congenital hypothyroidism occurs with a prevalence of approximately 1 in 4000 newborns [69,70]. Patients with this syndrome can be divided into two groups: nongoitrous (dysembryogenesis) and goitrous (dyshormonogenesis) congenital hypothyroidism. The dysembryogenesis or dysgenesis group, which accounts for 85% of the cases, results from ectopy thyroid tissue at the base of the tongue or in any position along the thyroglossal tract, agenesis and hypoplasia. In a minority of these patients, the congenital hypothyroidism is associated with mutations in genes responsible for the development or growth of thyroid follicular cells: thyroid transcription factor 1 (TTF-1, also known as TITF1, NKX2-1 or T/EBP) [71–73], thyroid transcription factor 2 (TTF-2, also known as TITF2, FOXE1 or FKHL15) [74], paired box transcription factor 8 (PAX-8) [75–78], thyrotrophin (TSH) [79,80] and TSH receptor genes [81–83]. The presence of congenital goiter (which accounts for the remaining 15% of the cases) has been linked to mutations in the NIS [84–86], Tg [46–65], TPO [87–93], DUOX 2 [94–96] and PDS genes [97,98]. These mutations originate a heterogenous spectrum of congenital goitrous, transmitted in an autosomal recessive mode. The patients with Tg synthesis defects present a congenital goiter, hypothyroidism or euthyroidism, high iodide ^{131}I uptake, normal organification of iodide, elevated serum TSH with simultaneous low or normal serum T_4 and T_3 levels, and low serum Tg concentration in relation to the degree of TSH stimulation [69,70]. Neonatal thyroid screening programs have been largely successful in diagnosis and treatment of congenital hypothyroidism. The early identification and treatment of congenital hypothyroidism are effective in preventing mental retardation and the other long-term consequences of hypothyroidism in most cases.

The Tg gene has been identified as the major susceptibility gene for familial autoimmune thyroid diseases (AITD), by linkage analysis using highly informative polymorphic markers [99–105].

This paper reviews the recently advances in our knowledge of Tg disorders, in man and animals, their pattern of inheritance, genetic markers, mutational processes and pathogenesis mechanism, with special emphasis on the genetic mechanism responsible for congenital goiter.

2. The nucleotide and amino acid nomenclatures

The nucleotide position in human Tg mRNA is designated according to 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 have negative numbering, while those located downstream have positive numbering.

3. The thyroglobulin gene: structure, expression and regulation

Tg is a homodimeric glycoprotein of 660 kDa synthesized and secreted by the thyroid cells into the follicular lumen. Tg is synthesized as a 12 S molecule, but forms 19 S homodimers and even 27 S tetramers. In human, it is coded by a single copy gene, 270 kb long (Table 1) [36,38–40], that maps on chromosome 8q24.2–8q24.3 [41–45] and contains an 8.5 kb coding sequences divided into 48 exons, separated by introns varying in size up to 64 kb (GenBank accession number NT_008046) [36,38–40]. The 64 kb intron (intron 41) of the Tg gene is an example of a large intron containing a small gene [106]. This gene codes for the human Src-like adaptor protein and appears to be transcribed in the opposite direction to Tg.

Tg gene expression is controlled positively by TSH through the modulation of the intracellular level of cyclic adenosine monophosphate (cAMP) via its receptor (TSHr) located at the basal membrane of the cell [107–117]. Transcription of the Tg gene is regulated by thyroid-specific transcription factors TTF-1 [118,119], TTF-2 [120–122] and PAX-8 [123]. It is mediated by binding to the Tg promoter on their consensus sequences [124–126].

The human Tg mRNA is 8449–8468 kb long [127–131]. The general organization of the sequence showed a 41-nucleotide 5′-untranslated segment, followed by a single open reading frame of 8307 bases and a 3′-untranslated segment ranging from 101 up to 120 bp. Tg mRNA in human thyroid tissues is very heterogeneous due to 21 nucleotide polymorphisms [56,65,130–132], 11 alternatively spliced transcripts [130,133–137] and 4 polyadenylation cleavage site variants [132]. The preprotein monomer is composed of a 19-amino acid signal peptide followed by a 2749 residues polypeptide [129,132] (Fig. 2). The 80% of the monomer primary structure is characterized by the presence of three types of repetitive units [129,132]. The remaining 20%, that constitutes the carboxy-terminal domain of the molecule, is not repetitive and shows a striking homology with acetylcholinesterase (ACHE) [138,139]. This suggests a probable convergent origin of the Tg gene from different ancestral DNA sequences. The relation between the three families of cysteine-rich repetitive units and the intron–exon junctions organization was analyzed [40]. The monomer contains eleven Type 1, three Type 2 and five Type 3 repeat motifs (Fig. 2). The analysis in detail of the repeats showed the following distribution: (i) Repeats Type 1, 2, 4, 7, 10 and 11 are each encoded by a single exon (exon 4, 8, 10, 16 and 22, respectively), repeats 1 and 9 by two exons (exon 2 and 3, and 14 and 15, respectively), repeats 3 and 8 by three exons (exon 5, 6 and 7 and 11, 12 and 13, respectively) and repeats 5 and 6 are a fraction of exon 9. (ii) The three Type 2 repetitive elements map between exons 20 and 21. (iii) The Type 3 domain includes two subtypes, 3a and 3b, and map between exons 23 and 37 (3a-1: between exons 23 and 26, 3b-1: between exons 26 and 30, 3a-2:

Table 1
Intron–exon organization of the human thyroglobulin gene

3′ end intronic sequences	Exon 5′ end	Exon number	Exon size (bp)	Exon 3′ end	5′ end intronic sequences	Intron number	Intron size (bp)
	5′UTR	1	108	TTC G	gtaagt	1	1047
ttttcttttcctag	AG TAC	2	109	TTC CA	gtaagg	2	1505
tgtctcctcctcag	G ACT	3	98	GCT T	gtaagt	3	1521
ccttgtacccacag	GT CTG	4	204	CGA T	gtgagt	4	1510
gtgaaaatgtttag	GT CCA	5	160	AAC AG	gtaagg	5	8641
tcattctctccaag	G TTT	6	107	ACA G	gtgagt	6	499
ctgtctttgctcag	GT TTG	7	144	CGA T	gtaagt	7	201
tggatttcctctag	GC CCC	8	186	TGT G	gtgggt	8	3448
tttgtctcatgcag	CT GAA	9	1101	AAA T	gtaagt	9	435
ttgttcctccccag	GC CCC	10	585	ACA T	gtgagc	10	5121
ttttattcccctag	GT CCT	11	240	TCT A	gtgagt	11	3719
ttccctgactccag	CC TTA	12	138	ACT G	gtaagg	12	382
tggctcttttccag	GG CAC	13	78	CAG T	gtaagt	13	551
tctctctcccacag	GC CCG	14	113	CTA GAA	gtaagg	14	1326
cggctttgtctcag	ACA GGA	15	103	CAG T	gtgagt	15	1013
tgtctctgtgtcag	GC CCA	16	201	GAG A	gtaagt	16	5134
tttccttctcccag	GC CCG	17	213	CAA C	gtgagt	17	1285
gtgcttgcctgcag	GG CCC	18	155	ATC CAG	gtacat	18	3036
ctgtcttcttgtag	GTG AAG	19	157	ATT G	gtatgt	19	1513
cctgtgtcttacag	AG AGA	20	219	TGC G	gtaggt	20	6110
ctgtttttttctag	TT AAG	21	150	CAC T	gtaagt	21	3812
tctattggttctag	GT GTC	22	171	TTG A	gtaggt	22	5567
tgctttatttttag	TG ATG	23	117	ACA G	gtgagg	23	4368
catggtgcttgcag	AT TGC	24	116	GAC CAG	gtgagg	24	2079
ctttccatctccag	AAA CGA	25	109	AAG G	gtaggt	25	5486
tgcctttcccccag	GC CAA	26	192	GGA G	gtaatg	26	7233
cttgtgattctcag	GT GCC	27	168	AAG A	gtaagt	27	12064
atcttcctttgcag	GT CTG	28	66	AAA G	gtgagc	28	1920
ttttttcctcctag	AT TCT	29	81	GCA G	gtactg	29	3485
tgcttctttttcag	GT TTG	30	138	TCT C	gtaagt	30	1096
tctcttgcctgtag	GT TGT	31	177	AAA G	gtgagc	31	1487
cttcctctatgaag	TT ATA	32	112	AAT GG	gtaagc	32	2224
tcttcctatgccag	G TTC	33	80	AAA G	gtaata	33	724
tttttccaccccag	GA GGA	34	144	CCC A	gtaagt	34	10608
tttttccttttcag	TT GCT	35	63	AAA G	gtaagt	35	28488
gccttctctcctag	TG TCT	36	135	TCG G	gtaagg	36	1564
cctcttttctgcag	AA TGT	37	165	CCA G	gtaagc	37	4013
ccctttcccaacag	GA ATC	38	220	CCA AG	gtatgg	38	1604
gtctgtatctgcag	G GCC	39	94	AAT GTG	gtgagt	39	2295
tccaatacccacag	GCC CCT	40	160	TCT G	gtgagt	40	7670
cctcttttctgaag	GG TCC	41	203	CTG ATG	gtaagt	41	65019
gctttctcttccag	GGA GGC	42	165	ACC AAG	gtgagc	42	997
tgcatccaatgcag	CTC TTG	43	168	GTG AAG	gtaagc	43	17048
tttttttttctag	CAA TTT	44	182	ACC CG	gtaagc	44	3005
tctcttttcaccag	G GAC	45	108	GGC AG	gtaaga	45	15095
ctctgttttctcag	C CTG	46	135	TCA GG	gtaatt	46	1523
tctcatttgcccag	A AAT	47	191	GCA G	gtagca	47	1015
cccctctgtttcag	AT GGA	48	239	3′UTR			

Exons sequences are in capital letters, introns sequences are in lower-case letters.
Genomic sequence of the Tg gene: GeneBank data base accession numbers AH008122, AH007064, AF237421, AF255396, AH008090, AY053519, NT_008046.

between exons 30 and 33, 3b-2: between exons 33 and 36 and 3a-3: between exons 36 and 37). Type 1 repeats could function as binders and reversible inhibitors of the protease in the lysosomal pathway [140,141]. Once Tg has reached the follicular lumen, several tyrosine 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 (132) (Fig. 2) and several tyrosines localized at positions 130 (exon 4), 847 (exon 10) and 1448 (exon 21) have been proposed as outer ring donor sites [142]. Tyrosine 5 is the most likely acceptor site for the donated iodotyrosyl from positions 130 [143,144].

After translation, intensive posttranslational processes take place in the endoplasmic reticulum (ER), Golgi apparatus, apical membrane and follicular lumen and include homodimers assembly, intrachain disulfide bond formation, glycosylation, sialylation, sulphatation, phosphorylation, iodination and multimerization [145–149] (Fig. 1). Several ER chaperones, such as ERp72, calnexin, Grp94 and Bip, interact with Tg during its maturation

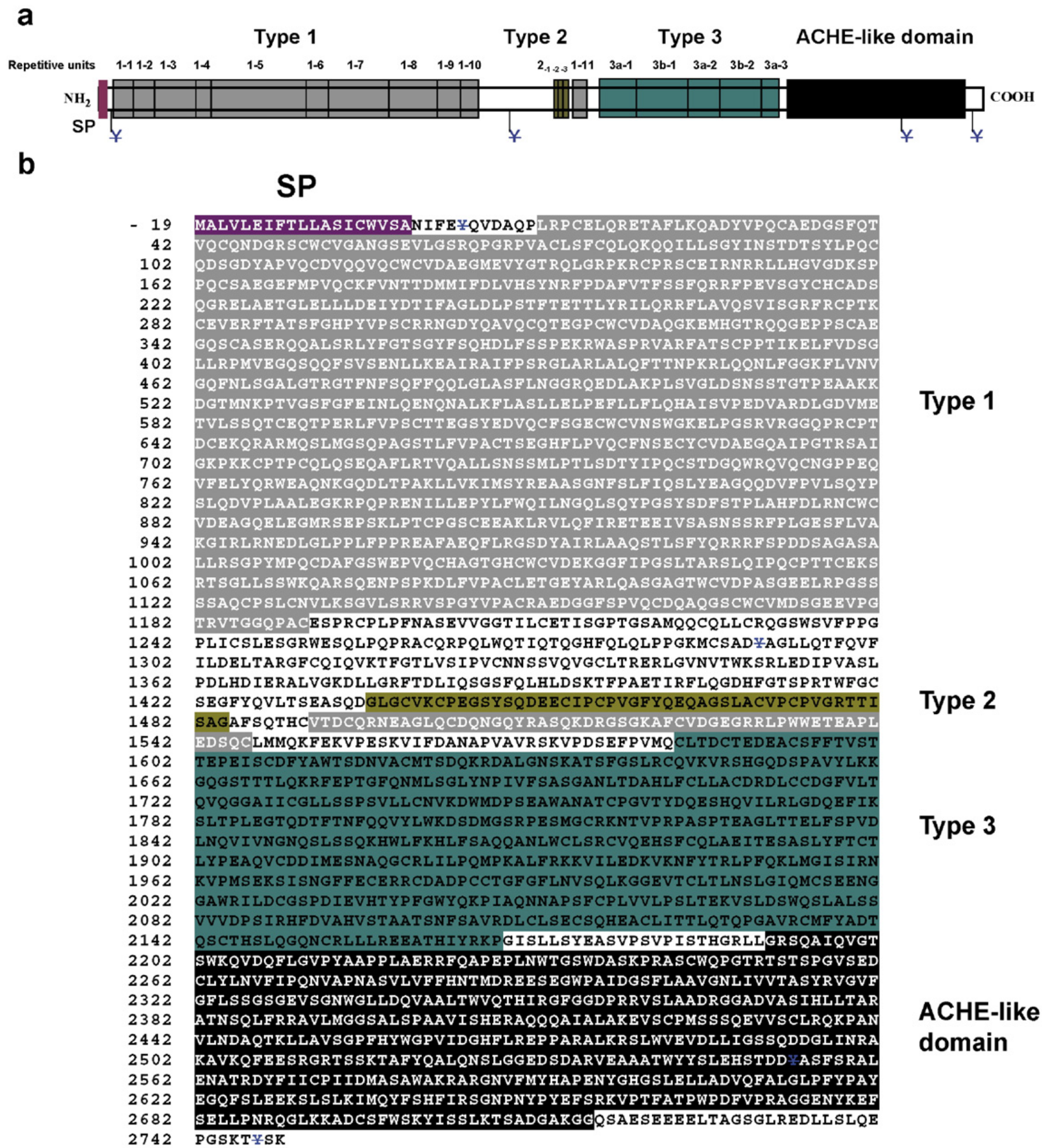

Fig. 2. Structural organization of the human thyroglobulin protein. a) Schematic representation of the repetitive, acetylcholinesterase-homology (ACHE-like domain) and hormonogenic domains. b) Wild-type protein sequence. The repetitive motifs (Type 1, Type 2 and Type 3), ACHE-like domain and hormonogenic sites are shown. ¥, tyrosine acceptor site; SP, signal peptide.

[52,150,151] and may serve to prevent the exportation of improperly folded Tg proteins. This process is known as ER quality control machinery [152].

On the other hand, Tg interacts with several proteins of the apical membrane in the exocytosis and endocytosis pathways of thyrocytes, such as Apical Membrane Asialoglycoprotein Receptor (ASGPR) [153], Megalin [154–157] and Protein Disulfide Isomerase (PDI) [158,159] (Fig. 1). The ASGPR transports new synthesized Tg to the follicular lumen. It is hypothesized that the ASGPR is also indirectly involved in the endocytosis and proteolytic cleavage of highly iodinated Tg. The region of the Tg that interacts with the receptor is unknown. The Tg regulates the thyroid gene expression mediating the ASGPR [153]. It is interesting to note that the follicular Tg acts as a feedback suppressor of the thyroid function [160,161], by inhibiting expression of TTF-1, TTF-2 and PAX-8 and consequently, decreasing expression of the Tg, TPO, NIS and TSHr genes. These findings support the idea that the Tg is not only the substrate for the biosynthesis of the thyroid hormones but also a regulator of the thyroid function, playing a role in transcriptional signaling or being related with some unknown mechanisms that remain to be determined.

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 mature Tg as a high affinity receptor for Tg [155].

Megalin interacts with a heparin-binding region (SRRLKRP) in the carboxyl-terminal portion of rat Tg [155]. However, this domain was not detected in human Tg when we searched the complete Tg protein for heparin-binding consensus sequences, using the computer program. Megalin plays a role in intact Tg transcytosis from apical to the basolateral surface of the thyrocyte [156,157] (Fig. 1). Subsequently, the endocytosis for proteolytic cleavage in the lysosomal pathway occurs via other mechanisms, such as fluid phase uptake or uptake by other affinity receptors.

In addition, it has been suggested that at the apical surface of the thyroid cell a quality control mechanism exists, that prevents premature lysosomal transfer and degradation of immature Tg [158]. The immature molecules are internalized and recycled through the trans-Golgi compartments [162]. PDI is thought to be the candidate receptor that mediates the internalization [159] (Fig. 1). However, there are no further reports to substantiate that observation.

To complete the complex profile of the Tg protein, the 11 most prominent antigenic regions were characterized using monoclonal antibodies [163,164]. These antigenic regions likely play a role in the correct positioning of hormonogenic tyrosines so as optimize iodination-coupling reactions [164].

4. Thyroglobulin gene molecular markers

Highly informative Tg DNA polymorphic markers were identified and can be used in linkage studies in families with congenital hypothyroidism or autoimmunity thyroid diseases. The Tg DNA polymorphisms proved to be interesting and informative genetic markers which also investigate whether a common ancestral chromosome or a mutational hot spot accounted for the occurrence of the same mutation in the all affected individuals. The term DNA polymorphism refers to a wide range of variations in nucleotide base composition, single nucleotide polymorphism (SNP), insertion and deletion sequences (Indel), or length of nucleotide repeats. This later group includes two categories of multiallelic tandemly repeated DNA sequences. Loci with repeated motifs of a few base pairs are often referred to as short tandem repeats (STR) or microsatellites, while those with longer repeated motifs are referred to as variable number of tandem repeats (VNTR) or minisatellites.

21 SNPs were identified and characterized in the coding sequence of the Tg gene, 14 of them resulting in amino acid polymorphisms: p.G58S, p.S715A, p.S715L, p.G796R, p.Q811E, p.R969P, p.M1009V, p.G1293D, p.T1479M, p.N1819D, p.R1980W, p.P2213L, p.W2482R, p.R2511Q [56,65,130–132] (Fig. 3, Table 2). There are no data on a putative functional role for these Tg changes.

A large insertion/deletion (Indel) polymorphism of 1464 bp localized in intron 18 of the human Tg gene was characterized [165]. Data from sequence showed a high A+T content (62%), a 17 bp-long motif (AAGAATTTTGGAGAACA) was found repeated two times, located at 791 and 849 bp downstream from exon 18 and three different types of 10 bp long palindromic sequences, ATTAGCTAAT, TTTTATAAAA and CAAA-TATTTG, were also found at positions 288, 870 and 1214, respectively. In addition, three short $(A)_n$ repeat traits along the sequence were identified. A Long PCR method was used to amplify the genomic DNA region containing intron 17/exon 18/intron 18/exon 19/intron 19 by primers situated in the introns 17 and 19. The amplification generates two fragments of 3.5 and 5.0 kb that correspond to the exclusion or inclusion of a 1464 bp segment, respectively. Both variants are thus widely represented in the human population; giving allele frequencies of 0.56 (insertion) and 0.44 (deletion). The Indel polymorphism was analyzed also by multiplex PCR. The amplification generates two fragments of 374 bp (between 18 exonic forward primer and 18 intronic reverse primer) and 541 bp (between 18 intronic forward primer and 18 intronic reverse primer, indicating the exclusion or inclusion of the Indel polymorphic region, respectively. Genetic evidence indicates that the small additions and deletions can occur spontaneously during replication. Deletion and insertion also result from recombination events or activities of the transposable elements. GenBank database search showed that the 1464 bp Indel polymorphism does not correspond to any known interspersed repetitive human sequence. However, it is not possible to exclude that some ancient transposable element, not identified in the intron 18, might have been involved in the development of this polymorphism.

STRs proved to be the most suitable markers in linkage analysis between a disease locus and a molecular marker, due to their diversity levels, high degree of resolution, relatively low mutation rates, high informativeness and rapid typing. Four STRs were identified and characterized within introns 10 (Tgms1), 27 (Tgms2), 29 (TGrI29) and 30 (TGrI30) of the Tg gene [100,166]. Tgms1 and Tgm2 consist of CA repeats and present 5 and 16 alleles, respectively. TGrI29 exhibited clearly 4 distinguishable alleles ranging from 197 to 203 bp in length and TGrI30 showed 8 alleles ranging from 502 to 542 bp. The heterozygosities (HET) observed of TGrI29 and TGrI30 were 0.859 and 0.522, respectively. The polymorphism information contents (PIC) were 0.471 and 0.434, respectively. No significant differences from Hardy–Weinberg values were found for these two systems. Sequencing analysis indicated that both loci are complex repeats, TGrI29 containing 2 types of variable motifs $(TC)_n$ and $(TG)_n$, and TGrI30 a tetra-nucleotide tandem units $(ATCC)_n$ [166]. In two TGrI29 alleles and one TGrI30 allele were found two different subtypes in each one, with the same molecular weights but different distribution of the tandem repeats.

The availability of highly informative polymorphic markers will allow indirect disease diagnosis by genetic linkage studies, such as in cases with no identified mutations and for rapid identification of affected newborns or gene carriers in families with Tg mutations.

5. Human thyroglobulin gene mutations

Thirty-five inactivating mutations have been identified and characterized in the human thyroglobulin gene: 20 missense mutations (p.C175G, p.Q310P, p.Q851H, p.S971I, p.R989C, p.P993L, p.C1058R, p.C1245R, p.S1447N, p.C1588F, p.C1878Y, p.I1912V, p.C1977S, p.C1987Y, p.C2135Y, p.R2223H, p. G2300D, p.R2317Q, p.G2355V, p.G2356R), 8 splice site

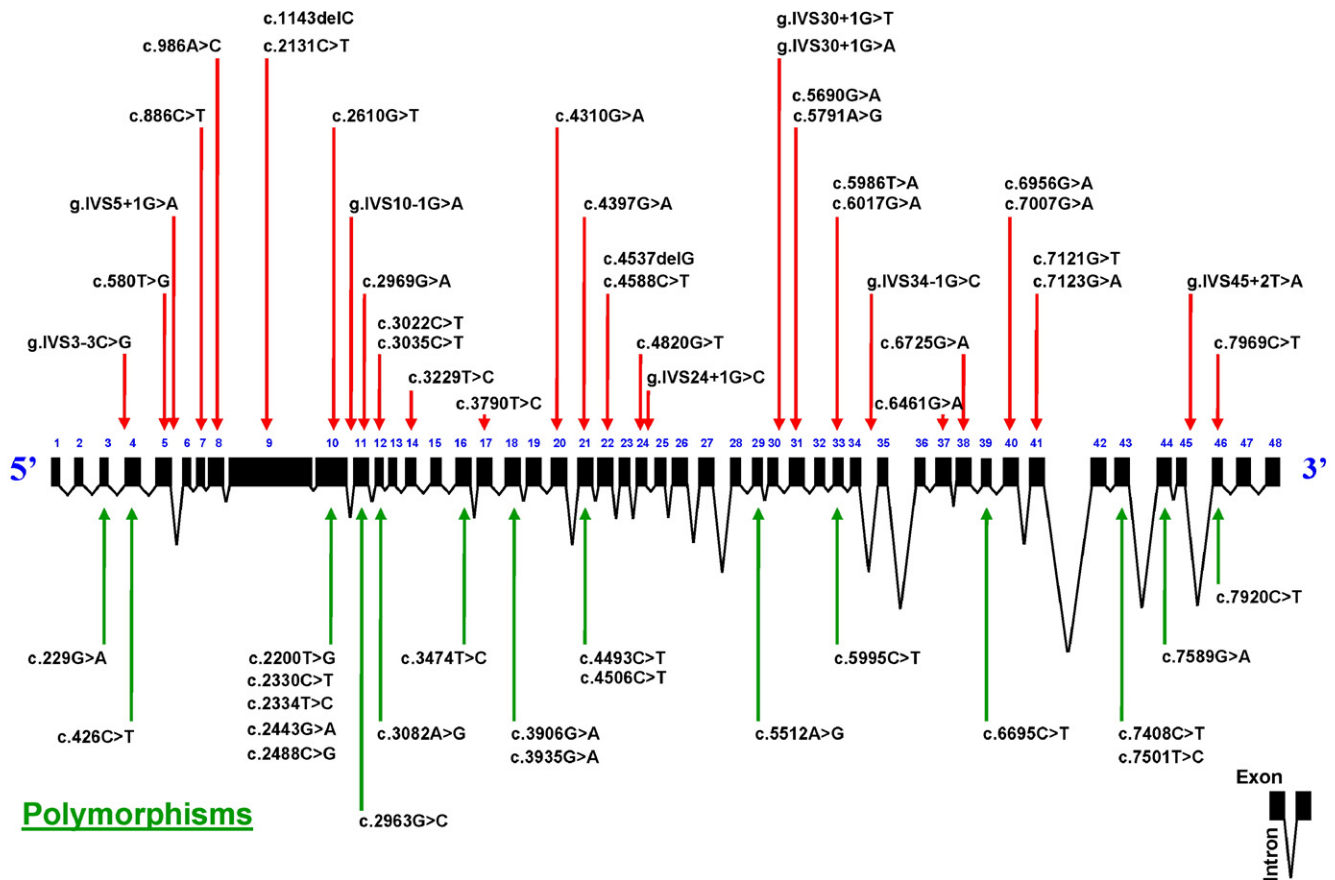

Fig. 3. Inactivating mutations and polymorphisms in the human thyroglobulin gene. Inactivating mutations and polymorphisms at the nucleotide level are described denoting the A of the initiator ATG as +1. Note the difference between scales used for introns and exons.

mutations (g.IVS3−3C>G, g.IVS5+1G>A, g.IVS10−1G>A, g.IVS24+1G>C, g.IVS30+1G>T, g.IVS30+1G>A, g.IVS34−1G>C, g.IVS45+2T>A) 5 nonsense mutations (p.R277X, p.Q692X, p.W1418X, p.R1511X, p.Q2638X) and two single nucleotide deletions (p.G362fsX382, p.D1494fsX1547) [46–65]. Fig. 3 and Table 3 summarize the reported inactivating mutations indicating the location of the mutation within the coding sequence or in the introns, and the amino acid alterations in the mature polypeptide.

In one such patient there was a defective synthesis of Tg due to the absence of exon 4 from the major Tg transcript because of a cytosine to guanine transversion at position minus 3 in the acceptor splice site of intron 3 (g.IVS3−3C>G) [46]. Removal of exon 4 does not modify the reading frame of Tg mRNA, producing an abnormal Tg devoid of the 68 residues. Exon 4 encodes tyrosine 130 which has been proposed as an important donor tyrosine involved in the synthesis of thyroxine, after coupling with the major acceptor tyrosine at position 5 [142–144]. The deletion is localized in the Tg Type 1 repeat domain.

An 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 family with a history of congenital goiter [51,57]. Both affected patients are homozygous for the mutation. The elimination of 138 nt 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. 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 [52] or could impair the coupling reaction. However, small amounts of functionally active Tg could be iodinated, and immediately hydrolized, yielding mostly T3, because of the intense tissue stimulation by TSH. The deletion is localized in the TG type III repeat domain, causing the loss of 1 putative N-linked glycosylation site.

A non-consanguineous Brazilian family with two affected siblings and a nephew presenting congenital goiter, hypothyroidism, and marked impairment of Tg synthesis was extensively studied by Targovnik et al. [47,49,53,69,61]. Molecular studies indicated that the affected individuals are either compound heterozygous for p.R277X/g.IVS34−1G>C or p.R277X/p.R1511X [59]. The p.R277X mutation (c.886C>T) in exon 7 is the most frequently reported mutation in the Tg gene [55,59,60]. Recently, a new case of congenital goiter with hypothyroidism caused by a homozygous p.R277X was reported [48,60]. The

Table 2
List of human thyroglobulin gene polymorphism

Exon	Nucleotide position	Amino acid position
3	c.229G>A	p.G58S
4	c.426C>T	p.D123D
10	c.2200T>G	p.S715A
10	c.2330C>T	p.P758L
10	c.2334T>C	p.P759P
10	c.2443G>A	p.G796R
10	c.2488C>G	p.Q811E
11	c.2963G>C	p.R969P
12	c.3082A>G	p.M1009V
16	c.3474T>C	p.S1139S
18	c.3906G>A	p.P1283P
18	c.3935G>A	p.G1293D
21	c.4493C>T	p.T1479M
21	c.4506C>T	p.A1483A
29	c.5512A>G	p.N1819D
33	c.5995C>T	p.R1980W
38	c.6695C>T	p.P2213L
43	c.7408C>T	p.L2451L
43	c.7501T>C	p.W2482R
44	c.7589G>A	p.R2511Q
46	c.7920C>T	p.Y2621Y

References: [56,65,130–132]. 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.

comparison of the Tg polymorphic markers identified in this patient with a member of the Brazilian family, who also carry the mutation as a compound heterozygous mutation [59], revealed 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 [60]. The truncated p.R277X form of Tg still harbors both the acceptor tyrosine 5 and the donor tyrosine 130 [143,144]. Consequently, a 26 kDa aminoterminus Tg peptide retained its ability for T4 synthesis. In agreement with this observation it should be considered that the small p.R277X peptide is sufficient for the synthesis of T_4 in the N-terminal domain. However, the premature stop codon eliminates the carboxy-terminal hormonogenic domain, resulting in the loss of thyroid hormone formation. The glycosylation of the Tg is an essential process that permits the migration from the ER to the Golgi. *In vitro* expression of the truncated p.R277X Tg cDNA showed that the mutated Tg protein can be glycosylated [55], indicating their possible exportation to apical surface of thyrocytes. The RT-PCR analysis [49,55,60] excluded an alternative splicing mechanism, by exon skipping, in order to restore the normal reading frame disrupted by the nonsense mutation and eliminate the stop codon which would truncate the protein.

The c.4588 C>T transition in exon 22 is also characterized by a predicted premature stop codon which results in a truncated protein of 1510 amino acids, p.R1511X [49,53,59,61]. However, the nonsense mutation is, thus, removed from the transcripts by exon skipping and there is a preferential accumulation in the goiter of a Tg mRNA lacking exon 22 [49,59]. Interestingly, 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 [135]. 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 alternative TG transcript lacking exon 22 constitutes a new case of nonsense-associated alternative splicing [61]. The construction and expression of the minigenes showed that the c.4588C>T mutation itself does not interfere with exon definition and processing *in vitro* [61]. The nonsense mutations in exon 7 and 22 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. The functional consequences of the deletion of exon 22 could be structural changes in the protein molecule that alter the normal protein folding and assembly, leading to a marked reduction in the ability to export the protein from the ER. Alternatively, it is possible that the elimination of exons containing repeat motifs by alternative splicing results in an altered ability to transfer an iodophenoxyl

Table 3
List of human thyroglobulin gene mutations

Exon/intron position	Nucleotide position	Amino acid position	References
Intron 3	g.IVS3−3C>G	Skipping of exon 4	[46]
Exon 5	c.580T>G	p.C175G	[65]
Intron 5	g.IVS5+1G>A	Skipping of exon 5	[64]
Exon 7	c.886C>T	p.R277X	[55,59,60]
Exon 8	c.986A>C	p.Q310P	[65]
Exon 9	c.1143delC	p.G362fsX382	[58]
Exon 9	c.2131C>T	p.Q692X	[65]
Exon 10	c.2610G>T	p.Q851H	[66,67]
Intron 10	g.IVS10−1G>A	Skipping of exon 11	[65]
Exon 11	c.2969G>A	p.S971I	[65]
Exon 12	c.3022C>T	p.R989C	[65]
Exon 12	c.3035C>T	p.P993L	[65]
Exon 14	c.3229T>C	p.C1058R	[62,65]
Exon 17	c.3790T>C	p.C1245R	[56,62,65]
Exon 20	c.4310G>A	p.W1418X	[65]
Exon 21	c.4397G>A	p.S1447N	[65]
Exon 22	c.4537delG	p.D1494fsX1547	[65]
Exon 22	c.4588C>T	p.R1511X Skipping of exon 22	[49,53,59,61]
Exon 24	c.4820G>T	p.C1588F	[65]
Intron 24	g.IVS24+1G>C	Skipping of exon 24	[65]
Intron 30	g.IVS30+1G>T	Skipping of exon 30	[51,57]
Intron 30	g.IVS30+1G>A	Skipping of exon 30	[65]
Exon 31	c.5690G>A	p.C1878Y	[63,65]
Exon 31	c.5791A>G	p.I1912V	[65]
Exon 33	c.5986T>A	p.C1977S	[56,62,65]
Exon 33	c.6017G>A	p.C1987Y	[65]
Intron 34	g.IVS34−1G>C	Skipping of exon 35	[59]
Exon 37	c.6461G>A	p.C2135Y	[65]
Exon 38	c.6725G>A	p.R2223H	[58]
Exon 40	c.6956G>A	p.G2300D	[65]
Exon 40	c.7007G>A	p.R2317Q	[63,65]
Exon 41	c.7121G>T	p.G2355V	[65]
Exon 41	c.7123G>A	p.G2356R	[62,65]
Intron 45	g.IVS45+2T>A	Skipping of exon 45	[65]
Exon 46	c.7969C>T	p.Q2638X	[65]

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.

group from the donor site to the acceptor iodotyrosine in the coupling machinery [59]. Cysteines are thought to play an important role in the tertiary structure of Tg, being five residues localized in exon 22. Exon 22 contains a tyrosine residue, at position 1510, that might be involved in hormonogenesis. The c.4588C>T mutation eliminates a restriction site for Taq I.

A third mutation was identified in the familiar group described above consisting of a guanine to cytosine transversion at position-1 in the acceptor site of intron 34 (g.IVS34−1G>C) [59]. This discovery infers the possibility that this splice site mutation might generate a total elimination of exon 35 of the Tg gene, since removal of 63 nucleotides maintains the reading frame. Exon 35 is the smallest exon of the TG gene and is flanked by two large introns (intron 34: 10,608 bp, intron 35: 28,488 bp). It is conceivable that intron size could affect the splicing. Gutnisky et al. [59] used an *in vitro* exon-trapping system to evaluate if the g.IVS34−1G>C mutation produces an abnormal transcript, by a defect in exon splicing. Minigenes were constructed using the pSPL3 vector which has a minimal gene organization: the SV40 promoter followed by an exon–intron–exon structure with a multiple cloning site (MCS) located inside the intron. When a fragment cloned in the MCS contains functional exons with their corresponding splicing sites, they are included in the mature mRNA. *In vitro* transcription showed that the mutation in the acceptor splice site causes the skipping of the exon 35. The excision of exon 35 in the Tg mRNA results in an in-frame deletion of 21-amino acid residues, which are located in the Tg Type 3 repeat motif [59].

A compound heterozygous mutation in the Tg gene was identified in a family with two affected siblings with congenital goitrous hypothyroidism [58]. 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). 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. The c.6725G>A mutation eliminates a restriction site for Hae II. The wild-type arginine residue at position 2223 is strictly conserved in all species for which suitable Tg and ACHE sequences have been reported [58]. 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 and the p.R2223H mutation may cause structural instability leading to deficient Tg exportation [58].

Important insights into the consequences of Tg alterations have been obtained from studies of various missense mutations. Two substitutions that replace cysteine by either arginine or serine (p.C1245R and p.C1977S) in exons 17 and 33, cause an abnormal three-dimensional structure of Tg resulting in defective intracellular transport of Tg and retention in the endoplasmic reticulum [56]. Kitanaka et al. [63] identified a patient with congenital goitrous hypothyroidism, who has high serum triodothyronine levels instead of low T4 and high TSH levels, due to a compound heterozygous mutation in the Tg gene. One of the mutations was a guanine to adenine transition at position 5690 in exon 31 (c.5690G>A), resulting in the substitution of cysteine by tyrosine in codon 1878 (p.C1878Y). The other was a guanine to adenine transition at position 7007 in exon 40 (c.7007G>A), resulting in the substitution of arginine by glutamine in codon 2317 (p.R2317Q).

Recent studies have shown that Tg gene mutations are associated with thyroid cancer development. Hishinuma et al. [62] reported patients with homozygous mutations (p.C1245R, p.C1977S and p.C1058R) or heterozygous compound (p.C1245R/p.G2356R). That alterations were missense mutations and were identified in papillary and follicular carcinoma, reflecting the fact that the incidence rate in patients with TG mutations was significantly higher. 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. On the other hand, Alzahrani et al. [64] identified a homozygous guanine to adenine point mutation at position +1 of the splice donor site of intron 5 (g.IVS5+1G>A) in Tg gene in a patient with recurrent goiter and a metastatic follicular thyroid carcinoma.

Recently, Hishinuma et al. [65] have reported 26 different inactivating mutations in the Tg gene in euthyroid to mildly hypothyroid, within the Japanese population, 20 are novel mutations: 12 missense mutations (p.C175G, p.Q310P, p.S971I, p.R989C, p.P993L, p.S1447N, p.C1588F, p.I1912V, p.C1987Y p.C2135Y, p.G2300D, p.G2355V), 4 splice mutations (g.IVS10-1G>A, g.IVS24+1G>C, g.IVS30+1G>A, g.IVS45+2T>A), 3 nonsense mutations (p.Q692X, p.W1418X, p.Q2638X) and one single nucleotide deletion (p.D1494fsX1547) (Fig. 3, Table 3). The patients harboring the frequent mutations p.C1058R and p.C1977S showed the same combinations of the SNPs in the coding region of the Tg gene [65]. Consequently, this finding suggests that the occurrence of these mutations is due to a founder effect.

6. Animal thyroglobulin gene mutations

Tg synthesis defects have been described not only in men, but also in cattle [167–173], goats [174–177], sheep [178–183], mouse [184–186], rats [187–190] and Bongo antelopes [191]. Hypothyroidism linked to Tg mutations have been reported in Afrikander cattle (p.R697X) [192–195], Dutch goats (p.Y296X) [196–198], cog/cog mouse (p.L2263P) [199] and rdw rats (p.G2300R) [200–202] (Table 4).

The congenital goiter of Afrikander cattle is an autosomal recessive disease characterized by Tg synthesis defect. Translation of goiter Tg mRNA produced no normal Tg protein [192,193], products of 250 and 75 kDa antigenically related to Tg were identified. The full-length bovine Tg mRNA contained an open reading frame of 8307 nt, which consists of a signal peptide of 19 residues and a mature protein of 2750 residues [31]. RNA analysis from affected animals revealed both a shorter (7300 nt) and normal sized TG mRNAs (8431) [193,194]. This shorter mRNA exists in low abundance in normal thyroid tissue, suggesting that it is normally produced by alternative splicing [194]. The 7300 nt mRNA encodes the 250 kDa peptide, whether the presence of the 75 kDa peptide was caused for an in-frame stop codon in the full-length mRNA. The mutation responsible for the diseases is a cytosine to thymine transition at nucleotide position 2146 in exon 9 (c.2146C>T) that generates a stop codon at amino acid position

Table 4
List of thyroglobulin gene mutations in animal species

Species	Phenotype	Nucleotide positions	Amino acid positions	References
Afrikander cattle	Goiter, euthyroidism	c.2148C>T Nonsense-mediated exon skipping of exon 9	p.R697X	Tassi et al. [192], Ricketts et al. [193–195]
Dutch goat	Goiter, hypothyroidism	c.945C>G	p.Y296X	Sterk et al. [196], van Ommen et al. [197], Veenboer and de Vijlder [198]
cog/cog mouse	Goiter, hypothyroidism	c.6848T>C	p.L2263P	Kim et al. [199]
WIC-rdw rat	No goiter, hypothyroidism	c.6958G>C	p.G2300R	Hishinuma et al. [200], Kim et al. [201], Baryshev et al. [202]

The nucleotide position is designated according to Tg mRNA reference sequences. The A of the ATG of the initiator methionine codon is denoted nucleotide +1. The amino acid positions are numbered after subtracting the amino acid signal peptide.

697 (p.R697X) [195,203]. The nonsense mutation is thus removed from the transcripts by exon skipping, and there is a preferential accumulation in the goiter of a 7300 nt mRNA lacking exon 9. The original reading frame is maintained in the shorter mRNA, which, as it lacks the mutated exon, is translatable into a potentially functional protein [195]. As was observed in human congenital goiter due to the p.R1511X mutation in the Tg gene, the nonsense mutation at codon 697 is expected to cause a dramatic destabilization of the full-length mRNA as a consequence of its limited translatability [195]. The 7300 nt mRNA preexisting as a minor mRNA species in normal animals, is actively translated into a 250 kDa Tg protein and escapes from the destabilization as it does not contain the stop mutation.

An inbred Dutch goat strain with hereditary congenital hypothyroidism and goiter was studied by de Vijlder et al. [174–177,196–198]. Tg antigens are found at 0.01% of the normal amount, sedimenting in a region corresponding to 200 kDa [176]. The goitrous Tg mRNA is present in reduced amounts, 2.5–10%. About 1% of the full-length Tg mRNA amount is found in the endoplasmic reticulum [176]. Translation of TG mRNA isolated from goiter in a cell-free system resulted in a 35 kDa Tg polypeptide [196]. The hereditary Tg synthesis defect in Dutch goats is caused by a cytosine to guanine mutation at position 945 (c.945C>G) that changes a triplet TAC coding for thyrosine (amino acid 296) in exon 8 into a triplet TAG giving a termination signal (p.Y296X) [198].

The cog/cog trait originally appeared as a spontaneous autosomal recessive phenotype in the inbred AKR/J strain of mouse [184 185]. A severe congenital hypothyroidism with colloid deficient goiter along with abnormal growth and central nervous system development was observed in cog/cog mouse, suggesting a defect of the Tg synthesis [186]. Tg mRNA was abundant and showed a normal size, but the reduced level of Tg protein exhibited enhanced susceptibility to proteolysis. The full-length mouse Tg mRNA contained an open reading frame of 8298 nt, which consisted of a signal peptide of 20 residues and a mature protein of 2746 residues [199]. Kim et al. [199] identified a missense mutation, contained within the ACHE -like domain of the Tg coding sequence, as the molecular basis for congenital hypothyroid goiter in cog/cog mouse. The thymine at nucleotide 6848 was substituted by cytosine (g.6848T>C), generating the p.L2263P mutation in the mature cog Tg protein [199]. The wild-type leucine residue in this position is conserved in all species for which suitable Tg sequence has been reported. Expression studies indicated that cog Tg exhibits a severe defect in the exit from the ER, whether the correction of this missense mutation restores the normal Tg secretion [199].

The rdw rat is a hereditary hypothyroid variant derived from the Wistar–Imamichi strain [187,188]. In contrast to human patients and animal models of congenital hypothyroidism, the rdw rat presents a hypoplastic thyroid gland that was smaller than the normal control, despite of the elevated circulating levels of TSH and the reduced level of T_3 and T_4. The immunohistochemical analysis showed that Tg was detected at very low levels in the colloid lumen with a substantial quantity in the dilated ER, suggesting an impaired intracellular transport of Tg [189]. Protein analysis revealed markedly elevated expression of molecular chaperones, GRP94, GRP78 and hsp70 [190]. The full-length rat Tg mRNA contained an open reading frame of 8304 nt, which consists of a signal peptide of 20 residues and a mature protein of 2748 residues [200,201,204–209]. The homology of the rat Tg with the mouse, bovine, and human TG is 90%, 76%, and 78%, respectively, at the nucleotide level; and 90%, 71%, and 74%, respectively, at the amino acid level. The complete sequencing of the rdw rat Tg cDNA revealed a single nucleotide change, the guanine at nucleotide 6958 was substituted with cytosine (g.6958G>C) [200,201]. The corresponding amino acid substitution was glycine with arginine (p.G2300R) at a position in the ACHE-like domain which is highly conserved in other mammalian species. As in cog/cog mouse models of defective Tg trafficking, the rdw Tg was retained inside the ER in cells that were transfected with the rdw Tg cDNA. The functional consequences of the missense substitution from a smaller neutral amino acid, glycine, to a larger basically charged amino acid, arginine, may have caused a conformational change in the C terminal region of Tg protein.

Baryshev et al. [202] demonstrated that the p.G2300R mutation in the rdw rat, as well as the p.C1245R and p.C1977S mutations in the human [56] induces the unfolded protein response (UPR). UPR is an adaptive cellular reaction that regulates the protein folding capacity of the ER 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 activated processing of activating transcription factor 6 (ATF6) and general translational attenuation by PERKR-like ER kinase (PERK). 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 [202].

cog/cog mouse and rwd rat models showed many similarities, both are associated with normal sizes and amounts of the Tg gene

transcripts, increased susceptibility to proteolysis of the mutant full-length Tg proteins, decreased synthesis and impairment of intracellular transport and marked accumulation of the chaperones, causing the named thyroidal ER storage diseases (ERSD) [199]. The only one important difference between the two models is the size of their thyroid glands. In the cog/cog mouse, the constant TSH stimulation leads to the development of a goiter, whether the rdw mutation is associated with a hypoplastic thyroid gland.

7. Thyroglobulin gene mutations in patients with nonendemic and endemic goiter

Nonendemic goiter is defined as an enlargement of the thyroid gland that is not the result of an inflammatory or neoplastic process, and is not associated with hyperthyroidism or hypothyroidism, in most cases the cause is unknown. In contrast, the iodine deficient is the most relevant etiologic factor in endemic goiter.

Corral et al. [67] described three unrelated families with nonendemic goiter, transmitted in an autosomal dominant mode. Analysis by direct sequencing showed a guanine to thymine transversion at position 2610 in exon 10 (c.2610G>T), which replaces the wild-type glutamine at codon 851 by an histidine (p.Q851H) (Fig. 3, Table 3). The missense mutation was identified in heterozygous state in 25 of 56 members of the three families, 14 of the carriers had developed the disease. However, the putative function of this mutation in the goiter development is not yet clear. The p.Q851H mutation was not detected in the general population, suggesting that this change is not a polymorphism. Consequently, it is not possible to exclude that this substitution affects the protein expression, intracellular transport, or hormonogenesis. The p.Q851H mutation was also described in one case with endemic goiter [66].

A large heterozygous deletion that involves the promoter region and the 11 first exons of the Tg gene has been reported, linked to a nonendemic goiter [68].

8. Risk of non-medullary thyroid cancer associated to the p.R2511Q thyroglobulin gene polymorphism

Non-medullary thyroid cancer accounts 90% of all thyroid cancers. Matakidou et al. observed that the frequency of the R allele of p.R2511Q [131,132] was over-represented in non-medullary thyroid cancer cases, in two studied populations, compared with controls [210]. The odd ratios associated with heterozygosity and homozygosity for the R allele were 1.6 (95% confidence interval, 1.1–2.5) and 2.0 (95% confidence interval, 1.2–3.3), respectively. This substitution resides within the ACHE homologous domain of Tg [132]. However, the precise role of the Tg protein variants in non-medullary thyroid cancer remains to be established.

9. Thyroglobulin gene is an important susceptibility gene for autoimmune thyroid disease

The AITD are the most common human autoimmune diseases. There are two forms of AITD, Graves' diseases, in which the production of TSHr stimulates antibodies causing hyperthyroidism, and Hashimoto thyroiditis, leading to hypothyroidism. Hashimoto's thyroiditis is an organ specific disorder, in which the thyroid cells are selectively destroyed. Both forms are characterized by infiltration of the thyroid by T cells and production of antiTg and antiTPO antibodies. AITD are complex diseases caused by an interaction between immunomodulatory genes, thyroid autoantigen specific gene and environmental factors. Three immunomodulatory genes have been identified as susceptible markers of the disease: cytotoxic T lymphocyte-antigen 4 (CTLA4) [211], the HLA [212] and the CD40 genes [213,214]. The CTL4 is considered a down regulator of T cell function, playing a key role in autoimmunity.

Several predisposing loci have been mapped through the entire human genome in AITD [99,101]. The most important locus is the 8q24, which contains the Tg gene. The microsatellite inside intron 27 (Tgms2), was informative and it showed a significative association with AITD, suggesting that the Tg gene was the AITD susceptibility gene on 8q24 [100,102,105]. No significant association between AITD and the Indel Tg polymorphism was observed [103]. Moreover, genotyping studies in Caucasian subjects, using Tg SNPs, demonstrated that the exon 10–12 SNP cluster and the exon 33 SNP were significantly associated with AITD [103]. The analysis demonstrated also that the combination of these two SNP groups was more significantly associated with AITD. In addition, the exon 33 SNP showed strong evidence for interaction with HLA-DR3 in conferring susceptibility to Graves' disease [215]. Interestingly, there is a significant association between the exon 10 SNP haplotype and murine experimental autoimmune thyroiditis. Fifty percent of the mouse strains susceptible to thyroiditis had the haplotype Ser–Met–Thr for exon 10, whereas all of the mouse strains that were resistant to thyroiditis had the haplotype Asn–Val–Ile [103]. These observations confirm that the Tg gene is an important susceptibility gene for AITD and demonstrated that the Tg is directly involved in the genetic etiology of AITD, both in human and in mouse. In this sense, it is interesting to hypothesize that the variations of the Tg protein structure may predispose to AITD by changing its antigenicity or its interaction with HLA molecules [103]. However, no evidence for association of exons 10, 12 and 33 SNPs with AITD was observed in Caucasian subjects by Collins et al. [104].

10. Conclusions and perspectives

The Tg is not only the substrate for the biosynthesis of thyroid hormones but also a regulator of thyroid function, playing a role in transcriptional signaling or being involved in some unknown mechanisms that remain to be explored. The identification, in the near future, of additional mutations in the Tg gene may provide important insights into structure–function relationships and may expand our knowledge of the molecular basis of familial hypothyroid or euthyroid goiter, resulting in a rapid prenatal diagnosis, and prevention of fetal hypothyroidism. Because the p.R277X, p.C1058R, p.C1245R and p.C1977S mutations are the most frequently reported mutations in the Tg gene, it would be helpful to investigate further cases with familial hypothyroid or euthyroid goiter, regarding these mutations. The identification of the Tg as an AITD susceptibility gene will enhance the prediction of individuals that are at high risk of developing the

diseases, as well as to understand the pathogenesis in order to establish the prevention and the best treatment of the disease. Further studies in the field of gene expression, molecular evolution and population genetics are necessary for elucidating the additional biological roles of the Tg.

Acknowledgements

C. M. Rivolta and H. M. Targovnik are established investigators of the Argentine National Research Council (CONICET).

This work was supported by grants from the Universidad de Buenos Aires (B 057/2004), CONICET (5360/2005) and ANPCyT-FONCyT (05-08838/PICT 2000/2001, 21081/PICT 2004).

References

[1] Van Herle AJ, Vassart G, Dumont JE. Control of thyroglobulin synthesis and secretion. N Engl J Med 1979;301:239–49 and 307–14.
[2] Kopp P. Thyroid hormone synthesis: thyroid iodine metabolism. In: Braverman LE, Utiger RD, editors. Werner and Ingbar's The thyroid: a fundamental and clinical text. 9th ed. Philadelphia: Lippincott Williams and Wilkins; 2005. p. 52–76.
[3] Dunn JT, Dunn AD. Thyroglobulin: chemistry, biosynthesis, and proteolysis. In: Braverman LE, Utiger RD, editors. Werner and Ingbar's The thyroid: a fundamental and clinical text. 8th ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 91–104.
[4] Arvan P, Di Jeso B. Thyroglobulin structure, function, and biosynthesis. In: Braverman LE, Utiger RD, editors. Werner and Ingbar's The thyroid: a fundamental and clinical text. 9th ed. Philadelphia: Lippincott Williams and Wilkins; 2005. p. 77–95.
[5] Taurog A. Hormone synthesis. In: Braverman LE, Utiger RD, editors. Werner and Ingbar's The thyroid: a fundamental and clinical text. 8th ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 61–85.
[6] Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. Nature 1996;379:458–60.
[7] Coyle B, Coffey R, Armour JAL, et al. Pendred syndrome (goitre and sensorineural hearing loss) maps to chromosome 7 in the region containing the nonsyndromic deafness gene DFNB4. Nat Genet 1996;12:421–3.
[8] Sheffield VC, Kraiem Z, Beck JC, et al. Pendred syndrome maps to chromosome 7q21–34 and is caused by an intrinsic defect in thyroid iodine organification. Nat Genet 1996;12:424–6.
[9] Rodriguez AM, Perron B, Lacroix L, et al. Identification and characterization of a putative human iodide transporter located at the apical membrane of thyrocytes. J Clin Endocrinol Metab 2002;87:3500–3.
[10] Libert F, Ruel J, Ludgate M, et al. Thyroperoxidase, an autoantigen with a mosaic structure made of nuclear and mitochondrial gene modules. EMBO J 1987;6:4193–6.
[11] Kimura S, Kotani T, Mc Bride OW, et al. Human thyroid peroxidase: compñete cDNA and protein sequence, chromosome mapping, and identification of two alternately spliced mRNA. Proc Natl Acad Sci U S A 1987;84:5555–9.
[12] Kimura S, Hong Y-S, Kotani T, Othaki S, Kikkawa F. Structure of the human thyroid peroxidase gene : comparison and relationship to the human myeloperoxidase gene. Biochemistry 1989;28:4481–9.
[13] Dupuy C, Ohayon R, Valent A, Noel-Hudson MS, Deme D, Virion A. Purification of a novel flavoprotein involved in the thyroid NADPH oxidase. Cloning of the porcine and human cDNAs. J Biol Chem 1999;274:37265–9.
[14] De Deken X, Wang D, Many MC, et al. Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. J Biol Chem 2000;275:23227–33.
[15] Wang D, De Deken X, Milenkovic M, et al. Identification of a novel partner of Duox: EFP1, a thioredoxin-related protein. J Biol Chem 2005;280:3096–103.
[16] Vassart G. Specific synthesis of thyroglobulin on membrane-bound thyroid polysomes. FEBS Lett 1972;22:53–6.
[17] Vassart G, Brocas H, Nokin P, Dumont JE. Translation in *Xenopus* oocytes of thyroglobulin mRNA isolated by poly(U)-Sepharose affinity chromatography. Biochim Biophys Acta 1973;324:575–80.
[18] Vassart G, Dumont JE. Identification of polysomes synthesizing thyroglobulin. Eur J Biochem 1973;32:322–30.
[19] Vassart G, Lecocq RE, Brocas H, Dumont JE. Translation in *Xenopus* oocyte of messenger RNA coding for a beef heavy-chain thyroglobulin. Biochim Biophys Acta 1974;353:261–5.
[20] Vassart G, Brocas H, Lecocq R, Dumont JE. Thyroglobulin messenger RNA: translation of a 33-S mRNA into a peptide immunologically related to thyroglobulin. Eur J Biochem 1975;55:15–22.
[21] Dinsart C, Van Voorthuizen F, Vassart G. Reverse transcription of thyroglobulin 33-S mRNA. Eur J Biochem 1977;78:175–81.
[22] Vassart G, Verstreken L, Dinsart C. Molecular weight of thyroglobulin 33 S messenger RNA as determined by polyacrylamide gel electrophoresis in the presence of formamide. FEBS Lett 1977;79:15–8.
[23] Christophe D, Brocas H, Gannon F, de Martynoff G, Pays E, Vassart G. Molecular cloning of bovine thyroglobulin complementary DNA. Characterization of 2500-base-pair and 1900-base-pair fragments. Eur J Biochem 1980;111:419–23.
[24] de Martynoff G, Pays E, Vassart G. Synthesis of a full length DNA complementary to thyroglobulin 33 S messenger RNA. Biochem Biophys Res Commun 1980;93:645–53.
[25] Vassart G, Brocas H. Restriction mapping of synthetic thyroglobulin structural gene as a means of investigating thyroglobulin structure. Biochim Biophys Acta 1980;610:189–94.
[26] Christophe D, Mercken L, Brocas H, Pohl V, Vassart G. Molecular cloning of the 8000-base thyroglobulin structural gene. Eur J Biochem 1982;122:461–9.
[27] Mercken L, Simons MJ, Vassart G. The 5′-end of bovine thyroglobulin mRNA encodes a hormonogenic peptide. FEBS Lett 1982;49:285–7.
[28] Mercken L, Massaer M, Simons MJ, Swillens S, Vassart G. Identification of hormonogenic domains in the carboxyl terminal region of bovine thyroglobulin. Biochem Biophys Res Commun 1984;125:961–6.
[29] Rawitch AB, Mercken L, Hamilton JW, Vassart G. The structure of a naturally occurring 10 K polypeptide derived from the amino terminus of bovine thyroglobulin. Biochem Biophys Res Commun 1984;119: 335–42.
[30] Mercken L, Simons MJ, de Martynoff G, Swillens S, Vassart G. Presence of hormonogenic and repetitive domains in the first 930 amino acids of bovine thyroglobulin as deduced from the cDNA sequence. Eur J Biochem 1985;147:59–64.
[31] Mercken L, Simons MJ, Swillens S, Massaer M, Vassart G. Primary structure of bovine thyroglobulin deduced from the sequence of its 8431-base complementary DNA. Nature 1985;316:647–51.
[32] van Ommen GJB, Arnberg AC, Baas F, et al. The human thyroglobulin gene contains two 15–17 kb introns nears its 3′-end. Nucleic Acids Res 1983;11:2273–85.
[33] Targovnik HM, Pohl V, Christophe D, Cabrer B, Brocas H, Vassart G. Structural organization of the 5′ region of the human thyroglobulin gene. Eur J Biochem 1984;141:271–7.
[34] Christophe D, Cabrer B, Bacolla A, Targovnik H, Pohl V, Vassart G. An unusually long poly(purine)-poly(pyrimidine) sequence is located upstream from the human thyroglobulin gene. Nucleic Acids Res 1985;13:5127–44.
[35] Baas F, van Ommen G-JB, Bikker H, Arnberg AC, de Vijlder JJM. The human thyroglobulin gene is over 300 kb long and contains introns of up to 64 kb. Nucleic Acids Res 1986;14:5171–86.
[36] Parma J, Christophe D, Pohl V, Vassart G. Structural organization of the 5′ region of the thyroglobulin gene. Evidence for intron loss and "exonization" during evolution. J Mol Biol 1987;196:769–79.
[37] Targovnik H, Paz C, Corach D, Christophe D. The 5′ region of the human thyroglobulin gene contains members of the Alu family. Thyroid 1992;2:321–4.
[38] Mendive FM, Rivolta CM, Vassart G, Targovnik HM. Genomic organization of the 3′ region of the human thyroglobulin gene. Thyroid 1999;9:903–12.

[39] Moya CM, Mendive FM, Rivolta CM, Vassart G, Targovnik HM. Genomic organization of the 5′ region of the human thyroglobulin gene. Eur J Endocrinol 2000;143:789–98.
[40] Mendive FM, Rivolta CM, Moya CM, Vassart G, Targovnik HM. Genomic organization of the human thyroglobulin gene. The complete intron–exon structure. Eur J Endocrinol 2001;145:485–96.
[41] Brocas H, Szpirer J, Lebo RV, et al. The thyroglobulin gene resides on chromosome 8 in man and on chromosome 7 in the rat. Cytogenet Cell Genet 1985;39:150–3.
[42] Avvedimento VE, Di Lauro R, Monticelli A, et al. Mapping of human thyroglobulin gene on the long arm of chromosome 8 by in situ hybridization. Hum Genet 1985;71:163–6.
[43] Baas F, Bikker H, Geurts van Kessel A, et al. The human thyroglobulin gene: a polymorphic marker localized distal to c-myc on chromosome 8 band q24. Hum Genet 1985;69:138–45.
[44] Bergé-Lefranc J-L, Cartouzou G, Mattei M-G, Passage E, Malezet-Desmoulins C, Lissitzky S. Localization of the thyroglobulin gene by in situ hybridization to human chromosomes. Hum Genet 1985;69:28–31.
[45] Rabin M, Barker PE, Ruddle FH, Brocas H, Targovnik H, Vassart G. Proximity of thyroglobulin and c-myc genes on human chromosome 8. Somat Cell Mol Genet 1985;11:397–402.
[46] Ieiri T, Cochaux P, Targovnik HM, et al. A 3′ splice site mutation in the thyroglobulin gene responsible for congenital goiter with hypothyroidism. J Clin Invest 1991;88:1901–5.
[47] Targovnik H, Propato F, Varela V, et al. Low levels of thyroglobulin messenger ribonucleic acid in congenital goitrous hypothyroidism with defective thyroglobulin synthesis. J Clin Endocrinol Metab 1989;69:1137–47.
[48] Targovnik HM, Varela V, Juvenal GJ, et al. Differential levels of thyroid peroxidase and thyroglobulin messenger ribonucleic acids in congenital goiter with defective thyroglobulin synthesis. J Endocrinol Invest 1990;13:797–806.
[49] Targovnik HM, Medeiros-Neto G, Varela V, Cochaux P, Wajchenberg BL, Vassart G. A nonsense mutation causes human hereditary congenital goiter with preferential production of a 171-nucleotide-deleted thyroglobulin ribonucleic acid messenger. J Clin Endocrinol Metab 1993;77:210–5.
[50] Medeiros-Neto G, Targovnik HM, Vassart G. Defective thyroglobulin synthesis and secretion causing goiter and hypothyroidism. Endocr Rev 1993;14:165–83.
[51] Targovnik H, Vono J, Billerbeck AEC, et al. A 138-nucleotide deletion in the thyroglobulin ribonucleic acid messenger in a congenital goiter with defective thyroglobulin synthesis. J Clin Endocrinol Metab 1995;80:3356–60.
[52] Medeiros-Neto G, Kim PS, Yoo SE, et al. Congenital hypothyroid goiter with deficient thyroglobulin. Identification of an endoplasmic reticulum storage disease with induction of molecular chaperones. J Clin Invest 1996;98:2838–44.
[53] Targovnik HM, Frechtel GD, Mendive FM, et al. Evidence for the segregation of three different mutated alleles of the thyroglobulin gene in a family with congenital goiter and hypothyroidism. Thyroid 1998;8:291–7.
[54] van de Graaf SAR, Cammenga M, Ponne NJ, et al. The screening for mutations in the thyroglobulin cDNA from six patients with congenital hypothyroidism. Biochimie 1999;81:425–32.
[55] van de Graaf SAR, Ris-Stalpers C, Veenboer GJM, et al. A premature stopcodon in thyroglobulin mRNA results in familial goiter and moderate hypothyroidism. J Clin Endocrinol Metab 1999;84:2537–42.
[56] Hishinuma A, Takamatsu J, Ohyama Y, et al. Two novel cysteine substitutions (C1263R and C1995S) of thyroglobulin cause a defect in intracellular transport of thyroglobulin in patients with congenital goiter and the variant type of adenomatous goiter. J Clin Endocrinol Metab 1999;84:1438–44.
[57] Targovnik HM, Rivolta CM, Mendive FM, Moya CM, Medeiros-Neto G. Congenital goiter with hypothyroidism caused by a 5′ Splice Site mutation in the thyroglobulin gene. Thyroid 2001;11:685–90.
[58] Caron P, Moya CM, Malet D, et al. Compound heterozygous mutations in the thyroglobulin gene (1143delC and 6725G→A[R2223H]) resulting in fetal goitrous hypothyroidism. J Clin Endocrinol Metab 2003;88:3546–53.
[59] Gutnisky VJ, Moya CM, Rivolta CM, et al. Two distinct compound heterozygous constellation (R277X / IVS34-1G>C and R277X / R1511X) in the thyroglobulin (TG) gene in affected individuals of a brazilian kindred with congenital goiter and defective TG synthesis. J Clin Endocrinol Metab 2004;89:646–57.
[60] Rivolta CM, Moya CM, Gutnisky VJ, et al. A new case of congenital goiter with hypothyroidism due to a homozygous p.R277X mutation in the exon 7 of the thyroglobulin gene: a mutational hot spot could explain the recurrence of this mutation. J Clin Endocrinol Metab 2005;90: 3766–70.
[61] Mendive FM, Rivolta CM, González-Sarmiento R, Medeiros-Neto G, Targovnik HM. Nonsense-associated alternative splicing of the human thyroglobulin gene. Mol Diagn 2005;9:143–9.
[62] Hishinuma A, Fukata S, Kakudo K, Murata Y, Ieiri T. High incidence of thyroid cancer in long-standing goiters with thyroglobulin mutations. Thyroid 2005;15:1079–84.
[63] Kitanaka S, Takeda A, Sato U, Miki Y, Hishinuma A, Ieiri T, et al. A novel compound compound heterozygous mutation in the thyroglobulin gene resulting in congenital goitrous hypothyroidism with high serum triiodothyronine levels. J Hum Genet 2006;15:379–82.
[64] Alzahrani AS, Baitei EY, Zou M, Shi Y. Metastatic thyroid follicular carcinoma arising from congenital goiter due to a novel splice donor site mutation in the thyroglobulin gene. J Clin Endocrinol Metab 2006;91:740–6.
[65] Hishinuma A, Fukata S, Nishiyama S, Nishi Y, Oh-Ishi M, Murata Y, et al. Haplotype analysis reveals founder effects of thyroglobulin gene mutations C1058R and C1977S in Japan. J Clin Endocrinol Metab First published ahead of print May 23, 2006 as doi:10.1210/jc.2005–2702.
[66] Pérez-Centeno C, González-Sarmiento R, Mories MT, Corrales JJ, Miralles-García JM. Thyroglobulin exon 10 gene point mutation in a patient with endemic goiter. Thyroid 1996;6:423–7.
[67] Corral J, Martín C, Pérez R, et al. Thyroglobulin gene point mutation associated with non-endemic simple goitre. Lancet 1993;341:462–4.
[68] González-Sarmiento R, Corral J, Mories MT, Corrales JJ, Miguel-Velado E, Miralles-Garcia JM. Monoallelic deletion in the 5′ region of the thyroglobulin gene as a cause of sporadic nonendemic simple goiter. Thyroid 2001;11:789–93.
[69] Medeiros-Neto G, Knobel M, DeGroot LJ. Genetic disorders of the thyroid hormone system. In: Baxter John D, editor. Genetics in Endocrinology. Philadelphia: Lippincott Williams and Wilkins; 2002. p. 375–402.
[70] Knobel M, Medeiros-Neto G. An outline of inherited disorders of the thyroid hormone generating system. Thyroid 2003;13:771–801.
[71] Iwatani N, Mabe H, Devriendt K, Kodama M, Miike T. Deletion of NKX2.1 gene encoding thyroid transcription factor-1 in two siblings with hypothyroidism and respiratory failure. J Pediatr 2000;137:272–6.
[72] Pohlenz J, Dumitrescu A, Zundel D, et al. Partial deficiency of thyroid transcription factor 1 produces predominantly neurological defects in humans and mice. J Clin Invest 2002;109:469–73.
[73] Krude H, Schütz B, Biebermann H, et al. Choreoathetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. J Clin Invest 2002;109:475–80.
[74] Clifton-Bligh RJ, Wentworth JM, Heinz P, et al. Mutation of the gene encoding human TTF-2 associated with thyroid agenesis, cleft palate and choanal atresia. Nat Genet 1998;19:399–401.
[75] Macchia PE, Lapi P, Krude H, et al. PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. Nat Genet 1998;19:83–6.
[76] Vilain C, Rydlewski C, Duprez L, et al. Autosomal dominant trasmission of congenital thyroid hypoplasia due to loss-of-function mutation of PAX8. J Clin Endocrinol Metab 2001;86:234–8.
[77] Congdon T, Nguyen LQ, Nogueira CR, Habiby RL, Medeiros-Neto G, Kopp P. A novel mutation (Q40P) in PAX8 associated with congenital hypothyroidism and thyroid hypoplasia: evidence for phenotypic variability in mother and child. J Clin Endocrinol Metab 2001;86:3962–7.
[78] Meeus L, Gilbert B, Rydlewski C, et al. Characterization of a novel loss of function mutation of PAX8 in a familial case of congenital hypothyroidism with in-place, normal-sized thyroid. J Clin Endocrinol Metab 2004;89:4285–91.
[79] Pohlenz J, Dumitrescu A, Aumann U, et al. Congenital secondary hypothiroidism caused by exon skipping due to a homozygous donor

splice site mutation in the TSHβ-subunit gene. J Clin Endocrinol Metab 2002;87:336–9.

[80] Borck G, Topaloglu AK, Korsch E, et al. Four cases of congenital secondary hypothyroidism due to a splice site mutation in the thyrotropin-β gene: phenotypic variability and founder effect. J Clin Endocrinol Metab 2004;89:4136–41.

[81] Sunthornthepvarakul T, Gottschalk ME, Hayashi Y, Refetoff S. Resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. N Engl J Med 1995;332:155–60.

[82] Abramowicz MJ, Duprez L, Parma J, Vassart G, Heinrichs C. Familial congenital hypothyroidism due to inactivating mutation of the thyrotropin receptor causing profound hypoplasia of the thyroid gland. J Clin Invest 1997;99:3018–24.

[83] Tonacchera M, Agretti P, Pinchera A, et al. Congenital hypothyroidism with impaired thyroid response to thyrotropin (TSH) and absent circulating thyroglobulin: evidence for a new inactivating mutation of the TSH receptor gene. J Clin Endocrinol Metab 2000;85:1001–8.

[84] Miki K, Harada T, Miyai K, Takai S-I. Amino N. Congenital hypothyroidism caused by a mutation in the Na^+/I^- symporter. Nat Genet 1997;16:124–5.

[85] Pohlenz J, Medeiros-Neto G, Gross JL, Silveiro SP, Knobel M, Refetoff S. Hypothyroidism in a brazilian kindred due to iodide trapping defect caused by homozygous mutation in the sodium/iodide symporter gene. Biochem Biophys Res Commun 1997;240:488–91.

[86] Pohlenz J, Rosenthal IM, Weiss RE, Jhiang SM, Burant C, Refetoff S. Congenital hypothyroidism due to mutations in the sodium/iodide symporter. Identification of a nonsense mutation producing a downstream cryptic 3′ splice site. J Clin Invest 1998;101:1028–35.

[87] Abramowicz MJ, Targovnik HM, Varela V, et al. Identification of a Mutation in the Coding Sequence of the Human Thyroid Peroxidase Gene Causing Congenital Goiter. J Clin Invest 1992;90:1200–4.

[88] Bikker H, den Hartog MT, Baas F, Gons MH, Vulsma T, de Vijlder JJM. A 20 basepair duplication in the human thyroid peroxidase gene results in a total iodide organification defect and congenital hypothyroidism. J Clin Endocrinol Metab 1994;79:248–52.

[89] Bikker H, Vulsma T, Baas F, de Vijlder JJM. Identification of five novel inactivating mutations in the human thyroid peroxidase gene by denaturating gradient gel electrophoresis. Hum Mutat 1995;6:9–16.

[90] Bikker H, Waelkens JJJ, Bravenboer B, de Vijlder JJM. Congenital hypothyroidism caused by a premature termination signal in exon 10 of the human thyroid peroxidase gene. J Clin Endocrinol Metab 1996;81: 2076–79.

[91] Bakker B, Bikker H, Vulsma T, de Randamie JSE, Wiedijk BM, de Vijlder JJM. Two decades of screening for congenital hypothyroidism in the Netherlands : TPO gene mutations in total iodide organification defect (an update). J Clin Endocrinol Metab 2000;85:3708–12.

[92] Bakker B, Bikker H, Hennekam RCM, et al. Maternal isodisomy for chromosome 2p causing severe congenital hypothyroidism. J Clin Endocrinol Metab 2001;86:1164–8.

[93] Rivolta CM, Esperante SA, Gruñeiro-Papendick L, et al. Five novel inactivating mutations in the human thyroid peroxidase gene responsible for congenital goiter and iodide organification defect. Hum Mutat 2003;22:259.

[94] Moreno JC, Bikker H, Kempers MJE, et al. Inactiving mutations in the gene for thyroid oxidase 2 (THOX2) and congenital hypothyroidism. N Engl J Med 2002;347:95–102.

[95] Vigone MC, Fugazzola L, Zamproni I, et al. Persistent mild hypothyroidism associated with novel sequence variants of the DUOX2 gene in two siblings. Hum Mutat 2005;26:395.

[96] Varela V, Rivolta CM, Esperante SA, Gruñeiro-Papendieck G, Chiesa A, Targovnik HM. Three mutations (p.Q36H, p.G418fsX482, and g.IVS19-2A>C) in the dual oxidase 2 gene responsible for congenital goiter and iodide organification defect. Clin Chem 2006;52:182–91.

[97] Kopp P. Pendred syndrome and genetic defects in thyroid hormone synthesis. Rev Endocr Metab Disord 2000;1:109–21.

[98] Borck G, Roth C, Martiné U, Wildhardt G, Pohlenz J. Mutations in the PDS gene in german families with Pendred's syndrome: V138F is a founder mutation. J Clin Endocrinol Metab 2003;88:2916–21.

[99] Sakai K, Shirasawa S, Ishikawa N, et al. Identification of susceptibility loci for autoimmune thyroid disease to 5q31–q33 and Hashimoto's thyroiditis to 8q23–q24 by multipoint affected sib-pair linkage analysis in Japanese. Hum Mol Genet 2001;10:1379–86.

[100] Tomer Y, Greenberg DA, Concepcion E, Ban Y, Davies TF. Thyroglobulin is a thyroid specific gene for the familial autoimmune thyroid diseases. J Clin Endocrinol Metab 2002;87:404–7.

[101] Tomer Y, Ban Y, Concepcion E, et al. Common and unique susceptibility loci in Graves and Hashimoto diseases: results of whole-genome screening in a data set of 102 multiplex families. Am J Hum Genet 2003;73:736–47.

[102] Collins JE, Heward JM, Carr-Smith J, Daykin J, Franklyn JA, Gough SCL. Association of a rare thyroglobulin gene microsatellite variant with autoimmune thyroid disease. J Clin Endocrinol Metab 2003;88:5039–42.

[103] Ban Y, Greenberg DA, Concepcion E, Skrabanek L, Villanueva R, Tomer Y. Amino acid substitutions in the thyroglobulin gene are associated with susceptibility to human and murine autoinmmune thyroid disease. Proc Natl Acad Sci U S A 2003;100:15119–24.

[104] Collins JE, Heward JM, Howson JMM, et al. Common allelic variants of exons 10, 12 and 33 of the thyroglobulin gene are not associated with autoinmune thyroid disease in the United Kingdom. J Clin Endocrinol Metab 2004;89:6336–9.

[105] Ban Y, Tozaki T, Taniyama M, Tomita M, Ban Y. Asociation of a thyroglobulin gene polymorphism with Hashimoto's thyroiditis in the japanese population. Clin Endocrinol (Oxf) 2004;61:263–8.

[106] Meijerink PHS, Yanakiev P, Zorn I, et al. The gene for the human Src-like adaptor protein (hSLAP) is located within the 64-Kb intron of the thyroglobulin gene. Eur J Biochem 1998;254:297–303.

[107] Van Heuverswyn B, Streydio C, Brocas H, Refetoff S, Dumont J, Vassart G. Thyrotropin controls transcription of the thyroglobulin gene. Proc Natl Acad Sci U S A 1984;81:5941–5.

[108] Avvedimento VE, Tramontano D, Ursini MV, Monticelli A, Di Lauro R. The level of thyroglobulin mRNA is regulated by TSH both in vitro and in vivo. Biochem Biophys Res Commun 1984;122:472–7.

[109] Van Heuverswyn B, Leriche A, Van Sande J, Dumont JE, Vassart G. Transcriptional control of thyroglobulin gene expression by cyclic AMP. FEBS Lett 1985;188:192–6.

[110] Hansen C, Gerard C, Vassart G, Stordeur P, Christophe D. Thyroid-specific and cAMP-dependent hypersensitive regions in thyroglobulin gene chromatin. Eur J Biochem 1988;178:387–93.

[111] Christophe D, Gerard C, Juvenal G, et al. Identification of a cAMP-responsive region in thyroglobulin gene promoter. Mol Cell Endocrinol 1989;64:5–18.

[112] Hansen C, Javaux F, Juvenal G, Vassart G, Christophe D. cAMP-dependent binding of a trans-acting factor to the thyroglobulin promoter. Biochem Biophys Res Commun 1989;160:722–31.

[113] Parmentier M, Libert F, Maenhaut C, et al. Molecular cloning of the thyrotropin receptor. Science 1989;246:1620–2.

[114] Donda A, Vassart G, Christophe D. Isolation and characterization of the canine thyroglobulin gene promoter region. Biochim Biophys Acta 1991;1090:235–7.

[115] Christophe Hobertus C, Donda A, Javaux F, Vassart G, Christophe D. Identification of a transcriptional enhancer upstream from the bovine thyroglobulin gene. Mol Cell Endocrinol 1992;88:31–7.

[116] Berg V, Vassart G, Christophe D. A zinc-dependent DNA-binding activity co-operates with cAMPresponsive-element-binding protein to activate the human thyroglobulin enhancer. Biochem J 1997;323:349–57.

[117] Guazzi S, Price M, De Felice M, Damante G, Mattei MG, Di Lauro R. Thyroid nuclear factor 1 (TTF-1) contains a homeodomain and displays a novel DNA binding specificity. EMBO J 1990;9:3631–9.

[118] Javaux F, Bertaux F, Donda A, et al. Functional role of TTF-1 binding sites in bovine thyroglobulin promoter. FEBS Lett 1992;3:222–6.

[119] Hamdan H, Liu H, Li C, et al. Structure of the human NKX2.1 gene. Biochim Biophys Acta 1998;1396:336–48.

[120] Chadwick BP, Obermayr F, Frischauf AM. FKHL15, a new human member of the forkhead gene family located on chromosome 9q22. Genomics 1997;41:390–6.

[121] Zanini M, Avantaggiato V, Biffali E, et al. TTF-2, a new forkhead protein, shows a temporal expression in the developing thyroid which is

consistent with a role in controlling the onset of differentiation. EMBO J 1997;16:3185–97.
[122] Macchia PE, Mattei MG, Lapi P, Fenzi G, Di Lauro R. Cloning, chromosomal localization and identification of polymorphisms in the human thyroid transcription factor 2 gene (TITF2). Biochimie 1999;81:433–40.
[123] Poleev A, Fickenscher H, Mundlos S, et al. PAX 8, a human paired box gene: isolation and expression in the developing thyroid, kidney and Wilms' tumors. Development 1992;116:611–23.
[124] Sinclair AJ, Lonigro R, Civitareale D, Ghibelli L, Di Lauro R. The tissue-specific expression of the thyroglobulin gene requires interaction between thyroid-specific and ubiquitous factors. Eur J Biochem 1990;193: 311–8.
[125] Zannini M, Francis-Lang H, Plachov D, Di Lauro R. Pax-8, a paired domain-containing protein, binds to a sequence overlapping the recognition site of a homeodomain and activates transcription from two thyroid-specific promoters. Mol Cell Biol 1992;12:4230–41.
[126] Berg V, Vassart G, Christophe D. Identification of a thyroid-specific and cAMP-responsive enhancer in the upstream sequences of the human thyroglobulin promoter. Biochim Biophys Acta 1996;1307:35–8.
[127] Bergé-Lefranc J-L, Cartouzou G, Malthiery Y, Perrin F, JarryLissitzky B, Lissitzky S. Cloning of four DNA fragments complementary to human thyroglobulin messenger RNA. Eur J Biochem 1981;120:1–7.
[128] Brocas H, Christophe D, Pohl V, Vassart G. Cloning of human thyroglobulin complementary DNA. FEBS Lett 1982;137:189–92.
[129] Malthièry Y, Lissitzky S. Primary structure of human thyroglobulin deduced from the sequence of its 8448-base complementary DNA. Eur J Biochem 1987;165:491–8.
[130] van de Graaf SAR, Pauws E, de Vjilder JJM, Ris-Stalpers C. The revised 8307 base pair coding sequence of human thyroglobulin transiently expressed in eukaryotic cells. Eur J Endocrinol 1997;136:508–15.
[131] Mendive FM, Rossetti LC, Vassart G, Targovnik HM. Identification of a new thyroglobulin variant: a guanine-to-arginine transition resulting in the substitution of arginine 2510 by glutamine. Thyroid 1997;7:587–91.
[132] van de Graaf SAR, Ris-Stalpers C, Pauws E, Mendive FM, Targovnik HM, de Vijlder JJM. Up to date with human thyroglobulin. J Endocrinol 2001;170:307–21.
[133] Mercken L, Simons MJ, Brocas H, Vassart G. Alternative splicing may be responsible for heterogeneity of thyroglobulin structure. Biochimie 1989;71:223–6.
[134] Bertaux F, Noël M, Malthièry Y, Fragu P. Demonstration of a heterogeneous transcription pattern of thyroglobulin mRNA in human thyroid tissues. Biochem Biophys Res Commun 1991;178:586–92.
[135] Targovnik HM, Cochaux P, Corach D, Vassart G. Identification of a minor Tg mRNA transcript in RNA from normal and goitrous thyroids. Mol Cell Endocrinol 1992;84:R23–6.
[136] Mason ME, Dunn AD, Wortsman J, et al. Thyroids from siblings with Pendred's syndrome contain thyroglobulin messenger ribonucleic acid variants. J Clin Endocrinol Metab 1995;80:497–503.
[137] Bertaux F, Noël M, Lasmoles F, Fragu P. Identification of the exon structure and four alternative transcripts of the thyroglobulin-encoding gene. Gene 1995;156:297–301.
[138] Swillens S, Ludgate M, Mercken L, Dumont JE, Vassart G. Analysis and structure homologies between thyroglobulin and acetylcholinesterase: possible functional and clinical significance. Biochem Biophys Res Commun 1986;137:142–8.
[139] Park YN, Arvan P. The acetylcholinesterase homology region is essential for normal conformational maturation and secretion of thyroglobulin. J Biol Chem 2004;279:17085–9.
[140] Molina F, Pau B, Granier C. The Type 1 repeats of thyroglobulin regulate thyroglobulin degradation and T3, T4 release in thyrocytes. FEBS Lett 1996;391:229–31.
[141] Molina F, Bouanani M, Pau B, Granier C. Characterization of the Type 1 repeat from thyroglobulin, a cysteine-rich module found in proteins from different families. Eur J Biochem 1996;240:125–33.
[142] Lamas L, Anderson PC, Fox JW, Dunn JT. Consensus sequences for early iodination and hormonogenesis in human thyroglobulin. J Biol Chem 1989;264:13541–5.
[143] Palumbo G, Gentile F, Condorelli GL, Salvatore G. The earliest site of iodination in thyroglobulin is residue number 5. J Biol Chem 1990;265:8887–92.
[144] Dunn AD, Corsi CM, Myers HE, Dunn JT. Tyrosine 130 is an important outer ring donor for thyroxine formation in thyroglobulin. J Biol Chem 1998;273:25223–9.
[145] Kim PS, Arvan P. Folding and assembly of newly synthesized thyroglobulin occurs in a pre-golgi compartement. J Biol Chem 1991;266:12412–8.
[146] Sakurai S, Fogelfeld L, Schneider AB. Anionic carbohydrate groups of human thyroglobulin containing both phosphate and sulfate. Endocrinology 1991;129:915–20.
[147] Venot N, Nlend MC, Cauvi D, Chabaud O. The hormonogenic tyrosine 5 of porcine thyroglobulin is sulfated. Biochem Biophys Res Commun 2002;298:193–7.
[148] Yang S-X, Pollock HG, Rawitch AB. Glycosylation in human thyroglobulin: location of the N-linked oligosaccharide units and comparison with bovine thyroglobulin. Arch Biochem Biophys 1996;327:61–70.
[149] Berndorfer U, Wilms H, Herzog V. Multimerization of thyroglobulin (TG) during extracellular storage: isolation of highly crosslinked TG from human thyroids. J Clin Endocrinol Metab 1996;81:1918–26.
[150] Kim PS, Arvan P. Calnexin and BiP act as sequential molecular chaperones during thyroglobulin folding in the endoplasmic reticulum. J Cell Biol 1995;128:29–38.
[151] Kuznetsov G, Chen LB, Nigam SK. Several endoplasmatic reticulum stress proteins, including ERp72, interact with thyroglobulin during its maturation. J Biol Chem 1994;269:22990–5.
[152] Hammond C, Helenius A. Quality control in the secretory pathway. Curr Opin Cell Biol 1995;7:523–9.
[153] Ulianich L, Suzuki K, Mori A, et al. Follicular thyroglobulin (TG) suppression of thyroid-restricted genes involves the apical membrane asialoglycoprotein receptor and TG phosphorylation. J Biol Chem 1999;274:25099–107.
[154] Zheng G, Marino M, Zhao J, McCluskey RT. Megalin (gp330): a putative endocytic receptor for thyroglobulin. Endocrinology 1998;139:1462–5.
[155] Marino M, Friedlander JA, McCluskey RT, Andrews D. Identification of a heparin-binding region of rat thyroglobulin involved in megalin binding. J Biol Chem 1999;274:30377–86.
[156] Marino M, Zheng G, Chiovato L, et al. Role of megalin (gp330) in transcytosis of thyroglobulin by thyroid cells. A novelfunction in the control of thyroid hormone release. J Biol Chem 2000;275:7125–37.
[157] Marino M, Pinchera A, McCluskey RT, Chiovato L. Megalin in thyroid physiology and pathology. Thyroid 2001;11:47–56.
[158] Metzghrani H, Mziaut H, Courageot J, Oughideni R, Bastiani P, Miquelis R. Identification of the membrane receptor binding domain of thyroglobulin. Insights into quality control of thyroglobulin bioshynthesis. J Biol Chem 1997;272:23340–6.
[159] Metzghrani A, Courageot J, Mani JC, Pugniere M, Bastiani P, Miquelis R. Protein-disulfide isomerase (PDI) in FRTL5 cells. PH-dependet thyroglobulin/PDI interactions determine a novel PDI function in the post-endoplasmic reticulum of thyrocytes. J Biol Chem 2000;275:1920–9.
[160] Suzuki K, Lavaroni S, Mori A, et al. Autoregulation of thyroid-specific gene transcription by thyroglobulin. Proc Natl Acad Sci U S A 1998;95:8251–6.
[161] Suzuki K, Mori A, Lavaroni S, et al. In vivo expression of thyroid transcription factor-1 RNA and its relation to thyroid function and follicular heterogeneity: identification of follicular thyroglobulin as a feedback suppressor of thyroid transcription factor-1 RNA levels and thyroglobulin synthesis. Thyroid 1999;9:319–31.
[162] Miquelis R, Courageot J, Jacq A, Blanck O, Perrin C, Bastiani P. Intracellular routing of GlcNAc-bearing molecules in thyrocytes: selective recycling through the Golgi apparatus. J Cell Biol 1993;123:1695–706.
[163] Henry M, Zanelli E, Piechaczyk M, Pau B, Malthièry Y. A major human thyroglobulin epitope defined with monoclonal antibodies is mainly recognized by human autoantibodies. Eur J Immunol 1992;22: 315–9.
[164] Erregragui K, Prato S, Miquelis R, Barrande C, Daniel C, Fert V. Antigenic mapping of human thyroglobulin. Eur J Biochem 1997;244:801–9.
[165] Moya CM, Varela V, Rivolta CM, Mendive FM, Targovnik HM. Identification and characterization of a novel large insertion/deletion

polymorphism of 1464 base pair in the human thyroglobulin gene. Thyroid 2003;13:319–23.

[166] Rivolta CM, Moya CM, Mendive FM, Targovnik HM. Genotyping and characterization of two polymorphic microsatellite markers located within introns 29 and 30 of the human thyroglobulin gene. Thyroid 2002;12:773–9.

[167] Robbins J, van Zyl A, van der Walt K. Abnormal thyroglobulin in congenital goiter of cattle. Endocrinology 1966;78:1213–23.

[168] Theron CN, van Jaarsveld PP. The thyroidal serum iodoproteins in congenital bovine goitre. S Afr Med J 1972;46:756–62.

[169] Theron CN, van Jaarsveld PP. Immunochemical properties of a thyroglobulin-like 12 S iodoprotein in a congenital bovine goitre. S Afr Med J 1972;46:763–9.

[170] van Jaarsveld P, van der Walt B, Theron CN. Afrikander cattle congenital goiter: purification and partial identification of the complex iodoirotein pattern. Endocrinology 1972;91:470–82.

[171] Pammenter M, Albrecht N, Liedenbered W, van Taarsveld P. Afrikander cattle congenital goiter: characteristics of its morphology and iodoprotein pattern. Endocrinology 1978;102:954–65.

[172] Pammenter M, Bester A, Albrecht C, van Jaarsveld P. Afrikander cattle congenital goiter: characteristics of the defective 19 s thyroglobulin synthesis. Endocrinology 1979;104:1853–61.

[173] Ricketts MH, Schulz K, van Zyl A, et al. Autosomal recessive inheritance of congenital goiter in Afrikander cattle. J Hered 1985;76:12–6.

[174] Rijnberk A, de Vijlder JJM, van Dijk JE, Jorma TJ, Tegelaers WHH. Congenital defect in iodothyronine synthesis. Clinical aspects of iodine metabolism in goats with congenital goitre and hypothyroidism. Br Vet J 1977;133:495–503.

[175] De Vijlder JJ, van Voorthuizen WF, van Dijk JE, Rijnberk A, Tegelaers WH. Hereditary congenital goiter with thyroglobulin deficiency in a breed of goats. Endocrinology 1978;102:1214–22.

[176] Van Voorthuizen WF, Dinsart C, Flavell RA, DeVijlder JJ, Vassart G. Abnormal cellular localization of thyroglobulin mRNA associated with hereditary congenital goiter and thyroglobulin deficiency. Proc Natl Acad Sci U S A 1978;75:74–8.

[177] De Vijlder JJ, van Ommen GJ, Van Voorthuizen WF, et al. Nonfunctional thyroglobulin messenger RNA in goats with hereditary congenital goiter. J Mol Appl Genet 1981;1:51–9.

[178] Falconer IR. Studies of the congenitally goitrous sheep. Composition and metabolism of goitrous thyroid tissue. Biochem J 1966;100:197–203.

[179] Falconer IR. Studies of the congenitally goitrous sheep. The iodinated compounds of serum, and circulating thyroid-stimulating hormone. Biochem J 1966;100:190–6.

[180] Mayo GM, Mulhearn CJ. Inheritance of congenital goiter due to a thyroid defect in Merino Sheep. Aust J Agric Res 1969;20:533–47.

[181] Falconer IR, Roitt IM, Seamark RF, Torrigiani G. Studies of the congenitally goitrous sheep. Iodoproteins of the goiter. Biochem J 1970;117:417–24.

[182] Dolling CE, Good BF. Congenital goiter in sheep: isolation of the iodoproteins which replace thyroglobulin. J Endocrinol 1976;71:179–92.

[183] Jones BR, Greenway RM, Jolly RD, Labuc RH, David GB. A defect in thyroglobulin synthesis in an inherited ovine goiter: possible neonatal respiratory distress syndrome. N Z Vet J 1986;34:145–8.

[184] Beamer WG, Maltais LJ, DeBaets MH, Eicher EM. Inherited congenital goiter in mice. Endocrinology 1987;120:838–40.

[185] Taylor BA, Rowle L. The congenital goiter mutation is linked to the thyroglobulin gene in the mouse. Proc Natl Acad Sci U S A 1987;84: 1986–90.

[186] Basche M, Beamer WG, Schneider AB. Abnormal properties of thyroglobulin in mice with inherited congenital goiter (cog/cog). Endocrinology 1989;124:1822–9.

[187] Koto M, Sato T, Okamoto M, Adachi J. rdw rats, a new hereditary dwarf model in the rat. Jikken Dobutsu 1988;37:21–30.

[188] Umezu M, Kagabu S, Jiang J, Sato E. Evaluation and characterization of congenital hypothyroidism in rdw dwarf rats. Lab Anim Sci 1998;48:496–501.

[189] Sakai Y, Yamashina S, Furudate SI. Missing secretory granules, dilated endoplasmic reticulum, and nuclear dislocation in the thyroid gland of rdw rats with hereditary dwarfism. Anat Rec 2000;259:60–6.

[190] Oh-Ishi M, Omori A, Kwon JY, Agui T, Maeda T, Furudate SI. Detection and identification of proteins related to the hereditary dwarfism of the rdw rat. Endocrinology 1998;139:1288–99.

[191] Doi S, Shifrin S, Santisteban P, et al. Familial goiter in bongo antelope (*Tragelaphus eurycerus*). Endocrinology 1990;127:857–64.

[192] Tassi VPN, Di Lauro R, Van Jaarsveld P, Alvino CG. Two abnormal thyroglobulin like polypeptides are produced from Afrikander cattle congenital goitre mRNA. J Biol Chem 1984;259:10507–10.

[193] Ricketts MH, Vandenplas S, Van der walt M, van Jaarsveld PP, Bester AJ, Boyd CD. Afrikander cattle congenital goiter: size heterogeneity in thyroglobulin in mRNA. Biochem Biophys Res Commun 1985;126: 240–6.

[194] Ricketts MH, Pohl V, de Martynoff G, et al. Defective splicing of thyroglobulin gene transcripts in the congenital goitre of the Afrikander cattle. EMBO J 1985;4:731–7.

[195] Ricketts MH, Simons MJ, Parma J, Mercken L, Dong Q, Vassart G. A nonsense mutation causes hereditary goitre in the Afrikander cattle and unmasks alternative splicing of thyroglobulin transcripts. Proc Natl Acad Sci U S A 1987;84:3181–4.

[196] Sterk A, van Dijk JE, Veenboer GJ, Moorman AF, de Vijlder JJ. Normal-sized thyroglobulin messenger ribonucleic acid in Dutch goats with a thyroglobulin synthesis defect is translated into a 35,000 molecular weight N-terminal fragment. Endocrinology 1989;124: 477–83.

[197] van Ommen GJB, Sterk A, Merken LOY, Arnberg AC, Baas F, de Vijlder JJM. Studies on the structures of the normal and abnormal goat thyroglobulin genes. Biochimie 1989;71:211–21.

[198] Veenboer GJM, de Vijlder JJM. Molecular basis of the thyroglobulin synthesis defect in Dutch goats. Endocrinology 1993;132:377–81.

[199] Kim PS, Hossain SA, Park Y-N, Lee I, Yoo S-E, Arvan P. A single amino acid change in the acetylcholinesterase-like domain of thyroglobulin causes congenital goiter with hypothyroidism in the cog/cog mouse: A model of human endoplasmic reticulum storage diseases. Proc Natl Acad Sci U S A 1998;95:9909–13.

[200] Hishinuma A, Furudate S-I, Masamichi O-I, Nagakubo N, Namatame T, Ieiri T. A novel missense mutation (G2320R) in thyroglobulin causes hypothyroidism in rdw rats. Endocrinology 2000;141:4050–5.

[201] Kim PS, Ding M, Menon S, et al. A missense mutation G2320R in the thyroglobulin gene causes non-goitrous congenital primary hypothyroidism in the WIC-rdw rat. Mol Endocrinol 2000;14:1944–53.

[202] Baryshev M, Sargsyan E, Wallin G, et al. Unfolded protein response is involved in the pathology of human congenital hypothyroid goiter and rat non-goitrous congenital hypothyroidism. J Mol Endocrinol 2004;32:903–20.

[203] de Martynoff G, Pohl V, Mercken L, van Ommen GJ, Vassart G. Structural organization of the bovine thyroglobulin gene and of its 5′-flanking region. Eur J Biochem 1987;164:591–9.

[204] Brocas H, Christophe D, Van Heuverswijn B, Scherberg N, Vassart G. Molecular cloning of Pst I fragments from rat double stranded thyroglobulin complementary DNA. Biochem Biophys Res Commun 1980;96:1785–92.

[205] Di Lauro R, Obici S, Acquaviva AM, Alvino CG. Construction of recombinant plasmids containing rat thyroglobulin mRNA sequences. Gene 1982;19:117–25.

[206] Avvedimento EV, Musti AM, Obici S, Cocozza S, Di Lauro R. Structural organization of the 3′ half of the rat thyroglobulin gene. Nucleic Acids Res 1984;12:3461–72.

[207] Musti AM, Avvedimento EV, Polistana C, et al. The complete structure of the rat thyroglobulin gen. Proc Natl Acad Sci U S A 1986;83:323–7.

[208] Di Lauro R, Obici S, Condliffe D, et al. The sequence of 967 amino acids at the carboxyl-end of rat thyroglobulin. Location and surroundings of two thyroxine-forming sites. Eur J Biochem 1985;148:7–11.

[209] Formisano S, Moscatelli C, Zarrilli R, et al. Prediction of the secondary structure of the carboxy-terminal third of rat thyroglobulin. Biochem Biophys Res Commun 1985;133:766–72.

[210] Matakidou A, Hamel N, Popat S, et al. Risk of non-medullary thyroid cancer influenced by polymorphic variation in the thyroglobulin gene. Carcinogenesis 2004;25:369–73.

[211] Tomer Y, Greember DA, Barbesino G, Concepcion E, Davies TF. CTLA-4 and not CD28 is a susceptibility gene for thyroid autoantibody production. J Clin Endocrinol Metab 2001;86:1687–93.

[212] Stenszky V, Kozma L, Balazs C, Rochlitz S, Bear JC, Farid NR. The genetics of Graves' disease: HLA and disease susceptibility. J Clin Endocrinol Metab 1985;61:735–40.

[213] Tomer Y, Concepcion E, Greenberg DA. A C/T single-nucleotide polymorphism in the region of the CD40 gene is associated with Graves' disease. Thyroid 2002;12:1129–35.

[214] Kim TY, Park YJ, Hwang JK, et al. A C/T polymorphism in the 5′-untranslated region of the CD40 gene is associated with Graves' disease in Koreans. Thyroid 2003;13:919–26.

[215] Hodge SE, Ban Y, Strug LJ, et al. Possible interaction between HLA-DRbeta1 and thyroglobulin variants in Graves' disease. Thyroid 2006;16:351–5.