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Congenital Goiter with Hypothyroidism Caused by a 5′ Splice Site Mutation in the Thyroglobulin Gene

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In this work we have extended our initial molecular studies of a consanguineous family with two affected goitrous siblings (H.S.N. and Ac.S.N.) with defective thyroglobulin (Tg) synthesis and secretion because of a homozygotic deletion of a fragment of 138 nucleotides (nt) in the central region of the Tg mRNA, identified previously in H.S.N. In order to identify the intron/exon boundaries and to analyze the regions responsible for pre-mRNA processing corresponding to a 138 nt deletion, we performed a screening of a human genomic library. The intron/exon junction sequences were determined from one positive clone by sequencing both strands of the DNA template. The results showed that the deletion mapped between positions 5549 and 5686 of the Tg mRNA and corresponded to exon 30. The positions of the exon limits differed by three nucleotides from the previously reported data obtained from direct sequencing of the deleted reverse transcriptase-polymerase chain reaction fragment from H.S.N. These variations are because the intron/exon junctions in this region were not available at the time when the deletion was first described. The deletion 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 same 138 nt deletion was observed in reverse transcriptase-polymerase chain reaction studies performed in the thyroid tissues from Ac.S.N. Genomic DNA analysis showed that a G to T transversion was observed at position +1 in the donor site of intron 30. Both affected patients (H.S.N. and Ac.S.N.) are homozygous for the mutation whereas the normal sister (At.S.N.) had a normal allele pattern. The functional consequences of the deletion are related to structural changes in the protein molecule that either could modify the normal routing of the translation product through the membrane system of the cell or could impair the coupling reaction. Probably the mutant Tg polypeptide might be functionally active in the production of thyroid hormone, because in the presence of a normal iodine ingestion (~150 μg/day), Ac.S.N. was able to maintain normal serum levels of total triiodothyronine (T_3) associated with relatively low serum total thyroxine (T_4) with normal somatic development without signs of brain damage.

Introduction

THYROGLOBULIN (Tg) is a large glycoprotein that functions as the matrix for thyroid hormone synthesis and in storage of the inactive form of thyroid hormone and of iodine (1). The human Tg gene is a 300-kb unit containing 8.5 kb of exon material, localized in chromosome 8 (8q24.2—8q24.3) (1). The number of exons has been estimated as 48 (2). Several mutations in the Tg gene have been reported and are associated with congenital goiter and variable degrees of hypothyroidism (3–8).

Ieiri et al. (3) reported a hypothyroid woman with congenital goiter and marked impairment of Tg synthesis. Molecular studies revealed that exon 4 was missing from the major Tg transcript in the goiter and that this aberrant splicing was due to a C to G transversion at position −3 in the acceptor splice site of intron 3.

A cytosine to thymine transition creating a stop codon at position 1511 in exon 22 has been described in a study of family with congenital goiter (4). Two siblings were affected with goiter and hypothyroidism, whereas the father and three other siblings had normal thyroid function. However, were heterozygous for the nonsense mutation. This implies that an additional mutation must be present in the affected individuals, generating a compound heterozygote genotype (5).

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More recently, van de Graaf et al. (6) identified a C to T transition at nucleotide (nt) position 886 in exon 7, creating a stop codon at amino acid Arg 277 in three siblings from a consanguineous marriage, with goiter and a moderate degree of hypothyroidism. Additional analysis of the pedigree indicated that the homozygous nonsense mutation cosegregated with the clinical phenotype.

Another report has indicated that cysteine substitutions (C1245R and C1977S in exons 17 and 33, respectively) cause an abnormal three-dimensional structure of Tg and defective intracellular transport of Tg (7).

We have previously identified a 138-nt deletion in the Tg mRNA in one member of a family with a history of congenital goiter (8). The affected members (two siblings) had congenital goiter, variable degrees of hypothyroidism, and marked impairment of Tg synthesis. The diagnosis of defective Tg was based on low serum total thyroxine (T_4), low serum total triiodothyronine (T_3) (H.S.N.) or normal serum total T_3 (Ac.S.N.), a negative perchlorate discharge test, and the virtual absence of the serum Tg response to challenge by bovine thyrotropin (TSH).

In the present article, we extend our initial molecular studies to show that the affected members of this family have an aberrant splicing because of a G to T transvertion at position +1 in the donor splice site of intron 30.

Materials and Methods

Patients

This work was approved by the Ethical Committee of the Hospital das Clínicas, University of Sao Paulo Medical School, and informed consent was obtained from the patients.

A detailed clinical and laboratory evaluation of this consanguineous family has been reported previously (8). In brief, H.S.N., a 20-year-old male, had lived most of his life in an iodine-deficient area (Bahia), had congenital goiter and hypothyroidism, but seldom took the prescribed levothyroxine (LT_4) tablets. In spite of high levels of serum TSH and a multinodular goiter, serum Tg concentrations were below the limit of detection and did not increase after bovine TSH stimulation. Both siblings (H.S.N. and Ac.S.N.) underwent thyroidectomy because of compression symptoms caused by the presence of a large multinodular goiter. Only minute amounts of immunoassayable Tg-related antigens were detected by radioimmunometric assay in the goitrous tissue. Agarose gel electrophoresis of the denatured soluble protein fraction (SPF) confirmed the virtual absence of Tg in the thyroid gland. In both siblings, Tg mRNA was isolated from the goitrous tissues. Subsequently it was documented that H.S.N. had a 138-nt deletion in the central region of the Tg mRNA (8). His affected sister (Ac.S.N.), an 11-year-old goitrous girl, had only mild signs of hypothyroidism and developed normally. The family had moved from a small village in the state of Bahia to Sao Paulo when Ac.S.N. was approximately 2 years old. Therefore, for most of her life, As.S.N. had a normal level of iodine ingestion through constant use of iodized salt (40–100 mg/kg). Serum Tg basal levels were low (1.0 μg/L) with a small increase to 2.9 μg/L after bovine TSH stimulation.

Another clinically normal sister (At.S.N.), had normal thyroid function tests and normal serum Tg levels.

Screening of a human genome library

An aliquot of a human genomic library made of λ Dash II recombinant phages (Stratagene, La Jolla, Ca) was plated on *Escherichia coli* XL1-BlueMRA and screened by the filter replica method using reverse transcriptase-polymerase chain reaction (RT-PCR) fragments as hybridization probes: PCR 2.2, PCR 3.1, PCR 3.2, and PCR 3.3 (9). The probes mapping in the central region of the Tg mRNA (nt 3012–6010), according to new cDNA numbering. Prehybridization, hybridization, and washing of the filters were performed as described by Mendive et al. (2).

Screening of recombinant positive phages by PCR and DNA sequencing of recombinant phages

The DNA from aliquots of the each positive phage stocks, obtained in the screening by plate hybridization, was purified by proteinase K treatment and phenol extraction.

The polymerase chain reaction (PCR) was performed in 100 μL, using a standard PCR buffer (Gibco BRL, Life Technology, Gaithersburg, MD), containing 2.5 mM $MgCl_2$, 200 μM each dNTP (dATP, dCTP, dTTP, and dGTP), 4% dimethylsulfoxide, 2 units *Taq* polymerase (Gibco BRL) and 50 pmol of each reverse and forward primers. The samples were subjected to 40 cycles of amplification. Each cycle consisted of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, and primer extension at 72°C for 1 minute. The primers used for PCR amplification were: forward primer (EX 30 F): 5′ TGACAACAGAACTTTTCTCC 3′ and reverse primer (EX 30 R): 5′ acGAGAAAGGCACCATAGG 3′ (Fig. 1). The amplified products, 138 bp, were analyzed in a 2% agarose gel. The preparation of bacteriophage DNA was performed with Wizard lambda preps DNA purification system (Promega, Madison, WI).

The exon and intron/exon junctions DNA sequences were determined from λ phage clone isolated using the Taq polymerase-based chain terminator method (fmol, Promega) and the same exonic primers that were used for screening of recombinant positive phages by PCR.

RT-PCR amplification

Total RNA was prepared from human thyroid by the method of Chomczynski and Sacchi (10). The primers, amplified regions, and conditions used for RT-PCR were described previously (4,9).

Genomic PCR amplification and DNA sequencing

Leukocyte DNA was prepared by the sodium dodecyl sulfate (SDS)-proteinase K method. For this PCR reaction, the same PCR conditions as those described above were used. The primers used for PCR amplification were: forward primer (IN 29 F): 5′ gaactattcctgtctgaccc 3′ and reverse primer (IN 30 R): 5′ ccacagtgatccatgagttatgacac 3′ (Fig. 1).

The preparation of PCR amplification fragments was performed with Concert gel purification system (Gibco BRL). The exon and intron/exon junctions DNA sequences were determinated with the Taq polymerase-based chain terminator method (fmol, Promega) and the same intronic primers that were used for genomic PCR amplification.

FIG. 1. Nucleotide and deduced amino acid sequence of the thyroglobulin (Tg) gene exon 30 and intron 29/exon 30/intron 30 junction sequences. The exon mapped between positions 5549 and 5686 of the Tg mRNA. The exonic sequences are indicated by capital letters and the intronic sequences by lower-case letters. The positions of the amplification and sequence primers are underlined. *S* indicates undetermined nucleotide.

Results

Characterization of intron 29/exon 30/intron 30 junction

In order to identify the intron/exon boundaries and to analyze the regions responsible for pre-mRNA processing corresponding a 138-nt deletion in the Tg mRNA of H.S.N., we performed a screening of a human genomic library.

About 1.9×10^6 phages of a human genomic DNA library were screened by plaque hybridization with RT-PCR probes corresponding to 3 kb from the central region of the Tg mRNA. Fifty plaques were scored as positive. DNA was prepared from each recombinant positive phage stocks and exonic primers were used to perform PCR (see Materials and Methods). The predicted 138-bp amplified fragment was observed in 3 of the 16 stocks investigated and the intron/exon junction sequences were determined from one positive clone by sequencing both strands of the DNA template. The results showed that the 138-nt deletion mapped (Fig. 1) between positions 5549 and 5686 of the Tg mRNA and corresponded to exon 30. The positions of the exon limits differed by 3 nt from the previously reported data obtained from direct sequencing of the deleted RT-PCR fragment from H.S.N. (8). These variations are due to the intron/exon junctions in this region that contains the deletion of H.S.N. were not available at time when the deletion was first described.

RT-PCR analysis

The Tg mRNA from control and Ac.S.N.'s thyroid tissue were first reverse transcribed and then divided into several positions (16–8410) and the resulting complementary DNAs were amplified individually by PCR. Analysis by 1.5% agarose gel electrophoresis of the products showed the expected sizes in all amplified fragments, except for that corresponding to the region between positions 5127–6010 (PCR 3.3 fragment [9]) that lacked the normal 884-bp fragment. The approximate size of the amplified fragment was 750 bp. Sequencing of the smaller Ac.S.N. revealed that 138 bp were missing between positions 5549 and 5686 of the TG mRNA. Subsequently, we concluded that the region absent corresponded to exon 30. These results are in perfect agreement with the data obtained previously in the thyroid tissue of H.S.N. (8).

This deletion does not affect the reading frame of the resulting mRNA and is potentially fully translatable into a Tg polypeptide chain that is shorter by 46 residues. A glycine residue is maintained by the junction between the proximal G from glycine 1831 and the distal GT from arginine 1877. The predicted TG translation product is a madure protein of 2703 amino acids with an expected ~ 323,000 daltons.

Genomic DNA analysis

The splice sites bordering exon 30 were PCR amplified and sequenced from genomic DNA of the goitrous patients (H.S.N. and Ac.S.N.), their normal sister (At.S.N.), and one normal control. A G to T transversion (Fig. 2) was observed at position +1 in the donor site of intron 30 of the patients, as compared to the normal sequence (TT instead of GT). The sequence analysis showed that both patients, H.S.N. and Ac.S.N., are homozygous for the mutation, whereas the healthy sibling (At.S.N.) had the two normal alleles.

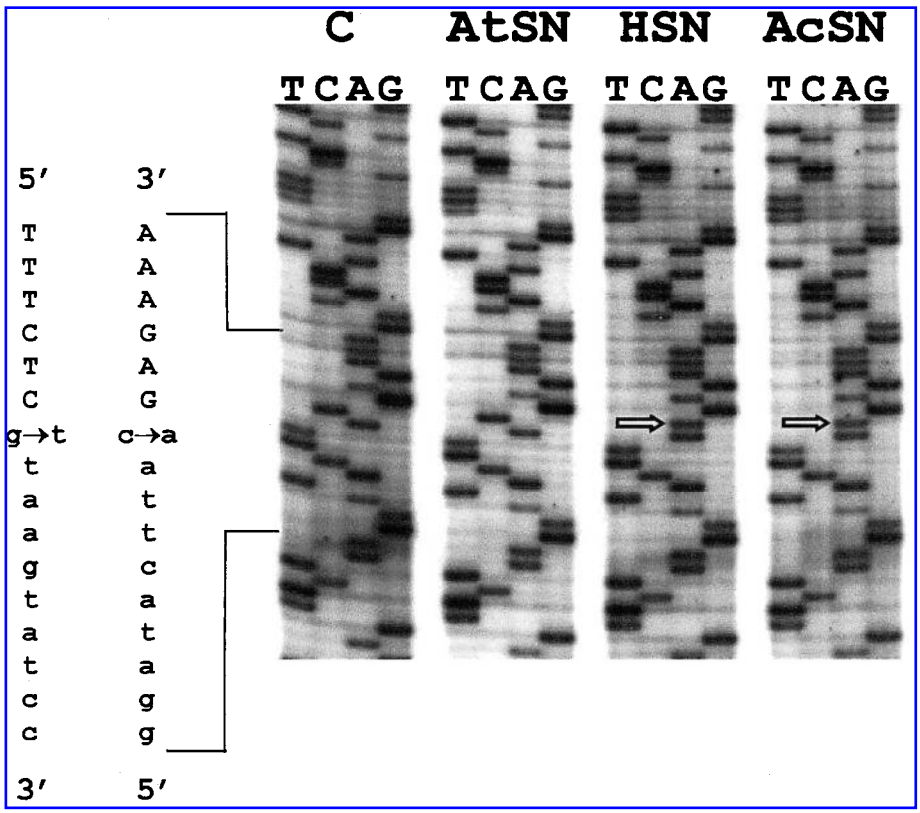

FIG. 2. Nucleotide sequences of the region of exon 30/intron 30 junction of the patients with congenital goiter showing the homozygous G → T transversion in position +1 in the donor site of intron 30. Sequence reactions were made with the same reverse primer (IN 30 R) that was used for genomic amplification. The arrows indicate the G → T transversion. C, normal control; At.S.N., sister with normal thyroid function; H.S.N. and Ac.S.N., affected individuals.

Discussion

The rapid accumulation of knowledge by the introduction of molecular biology in the field of thyroid genetic diseases has modified some physiopathological concepts and improved the diagnosis of resulting diseases. Prevalence of congenital hypothyroidism according to data from newborn screening programs is estimated to be 1 in 4,000 (ranging from 1 in 2,000 to 1 in 8,000). Patients with this heterogenous clinical and biochemical disorder can be divided into two groups: nongoitrous and goitrous neonatal hypothyroidism. Nongoitrous neonatal hypothyroidism results from thyroid gland agenesis (athyreosis), dysgenesis (ectopic gland), and hypoplasic thyroid gland. In most cases, the etiology is unknown. In a few patients, however, the congenital hypothyroidism is associated with mutations in genes responsible for the development of thyroid follicular cells: TSH-β (11), TTF-1 (12), PAX-8 (13), and TSH receptor genes (14). The presence of congenital goiter usually results from a number of abnormalities related to the protein components of thyroid hormonogenesis. Mutations in *NIS* symporter, Tg, thyroid peroxidase (TPO), and pendrin genes originate a wide spectrum of congenital goitrous hypothyroidism, mostly transmitted on the autosomal recessive mode (see Kopp [15] and references therein).

In the present study, we have identified a new mutation of the Tg gene in two siblings with congenital goitrous hypothyroidism. This kindred had been previously studied by us (8). The precise nature of the defect is a splicing error with total elimination of the exon 30 of the Tg gene, by a G to T transversion at position +1 in the donor site of intron 30. Homozygosity is consistent with a recessive trait. The precise excision of the 138 nt in the Tg mRNA results in an in-frame deletion of amino acid residues 1832–1877, which is localized in the Tg type III repeat domain (subtypes: a.2 and b.1 [2,3]), causing a loss of 1 putative N-linked glycosylation site. The functional consequences of the deletion are structural changes in the protein molecule that alter the normal routing of translation product through the membrane system of the cell (16,17). Alternatively, it may impair or substantially alter the coupling reaction. However, small amounts of functionally active Tg could be iodinated, and immediately hydrolized, yielding mostly T_3, because of the intense tissue stimulation by TSH.

A previous report suggests that these patients synthesize a mutant Tg that is defective for protein folding and assembly, leading to markedly reduced ability to export the protein from the endoplasmic reticulum with massive induction of selective synthesis of molecular chaperones, which bind to the misfolded exportable protein (17). Immunohistochemical localization of Tg data showed that the affected patients exhibit a marked decrease of reaction product in the

follicular lumina, with positive reaction product accumulated intracellularly in thyroid epithelial cells. Similar results were reported for the *cog/cog* mice (16) and human congenital goiters with other Tg mutations (7).

In addition to the defect in intracellular transport, the abnormalities in the coupling machinery may contribute to development of the congenital hypothyroid goiters in our patients. A well-organized three-dimensional Tg structure plays an important role in the coupling reaction that results in the fusion of two iodotyrosyl residues. Five hormonogenic acceptor tyrosines have been identified and localized at positions 5, 1291, 2554, 2568, and 2747 in human Tg and several tyrosines have been proposed as donor sites (18). It is acceptable that the deletion of 46 residues in the central region of Tg will affect its tertiary/quaternary structure implying in an altered ability to transfer an iodophenoxyl group from the donor site to the acceptor iodotyrosine in the coupling machinery.

The phenotype consequences of the defective Tg synthesis were not the same for H.S.N. and Ac.S.N. Although they shared the same abnormal mutant Tg, Ac.S.N. was only mildly hypothyroid and developed normally without mental retardation whereas H.S.N. had stunted growth, severe hypothyroidism, and clinical signs of brain damage (mental retardation). The major difference between the two siblings may be related to environmental conditions. Although Ac.S.N. lived most of her life in a iodine-sufficient environment (São Paulo), H.S.N. lived mostly in a low-iodine environment (a small village in Bahia). In goitrous humans with defective Tg synthesis (19) and in goitrous goats with a small mutant Tg transcript (20) excess iodine was able to restore euthyroidism in spite of marked structural changes in the mutant Tg. We concluded that the normal daily iodine supply (Ac.S.N.) was able to induce a normal supply of T_3 (but not T_4) that could maintain a certain degree of peripheral euthyroidism at cellular level. By contrast, H.S.N., who was raised in a low-iodine supply area, could not increase the synthesis of thyroid hormone in the mutant Tg. This could explain the different phenotype consequences for each sibling.

In conclusion, we were able to extend our initial studies in the defective Tg synthesis in this kindred with two affected siblings and a normal sister. In both congenitally goitrous and hypothyroid patients, a deletion of 138 nt was identified between positions 5549 and 5686 of the Tg mRNA corresponding to total elimination of exon 30. The resulting Tg polypeptide is shortened by 46 residues and may be partially functional for thyroid hormone synthesis in the presence of a normal iodine intake. The identification of an additional mutation in the Tg gene expands our knowledge of the molecular basis of congenital hypothyroidism and may provide, by direct sequencing of DNA amplified by PCR, a rapid prenatal diagnosis, and prevention of fetal hypothyroidism.

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References

1. Medeiros-Neto G, Targovnik HM, Vassart G 1993 Defective thyroglobulin synthesis and secretion causing goiter and hypothyroidism. Endocr Rev **14:**165–183.
2. Mendive FM, Rivolta CM, Vassart G, Targovnik HM 1999 Genomic organization of the 3′ region of the human thyroglobulin gene. Thyroid **9:**903–912.
3. Ieiri T, Cochaux P, Targovnik HM, Suzuki M, Shimoda S-I, Perret J, Vassart G 1991 A 3′ splice site mutation in the thyroglobulin gene responsible for congenital goiter with hypothyroidism. J Clin Invest **88:**1901–1905.
4. Targovnik HM, Medeiros-Neto G, Varela V, Cochaux P, Wajchenberg BL, Vassart G 1993 A nonsense mutation causes human hereditary congenital goiter with preferential production of a 171-nucleotide-deleted thyroglobulin ribonucleic acid messenger. J Clin Endocrinol Metab **77:**210–215.
5. Targovnik HM, Frechtel GD, Mendive FM, Vono J, Cochaux P, Vassart G, Medeiros-Neto G 1998 Evidence for the segregation of three different mutated alleles of the thyroglobulin gene in a Brazilian family with congenital goiter and hypothyroidism. Thyroid **8:**291–297.
6. van de Graaf SAR, Ris-Stalpers C, Veenboer GJM, Cammenga M, Santos C, Targovnik HM, de Vijlder JJM, Medeiros-Neto G 1999 A premature stopcodon in the thyroglobulin mRNA results in familial goiter and moderate hypothyroidism. J Clin Endocrinol Metab **84:**2537–2542.
7. Hishinuma A, Takamatsu J, Ohyama Y, Yokozawa T, Kanno Y, Kuma K, Yoshida S, Matsuura N, Ieiri T 1999 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 **84:**1438–1444.
8. Targovnik H, Vono J, Billerbeck AEC, Cerrone G, Varela V, Mendive F, Wajchenberg BL, Medeiros-Neto G 1995 A 138-nucleotide deletion in the thyroglobulin ribonucleic acid messenger in a congenital goiter with defective thyroglobulin synthesis. J Clin Endocrinol Metab **80:**3356–3360.
9. Moya CM, Mendive FM, Rivolta CM, Vassart G, Targovnik HM 2000 Genomic organization of the 5′ region of the human thyroglobulin gene. Eur J Endocrinol **143:**789–798.
10. Chomczynski P, Sacchi N 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem **162:**156–159.
11. Medeiros-Neto G, Herodotou DT, Rayan S, Kommerede S, Lacerca L, Sandrini R, Boguszewski MCS, Hollenberg AN, Rodovick S, Wondisford FE 1996 A circulating biologically inactive thyrotropin caused by a mutation in the beta subunit gene. J Clin Invest **97:**1250–1256.
12. Acebron A, Aza-Blanc P, Rossi DL, Lamas L, Santisteban P 1995 Congenital human thyroglobulin defect due to low ex-

pression of the thyroid-specific transcription factor TTF-1. J Clin Invest **96:**781–785.
13. Macchia PE, Lapi P, Krude H, Pirro MT, Missero C, Chiovato L, Souabni A, Baserga M, Tassi V, Pinchera A, Fenzi G, Gruters A, Busslinger M, Di Lauro R 1998 PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. Nat Genet **19:**83–86.
14. Sunthornthepvarakul T, Gottschalk ME, Hayashi Y, Refetoff S 1995 Resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. N Engl J Med **332:**155–160.
15. Kopp P 2000 Pendred's syndrome and genetic defects in thyroid hormone synthesis. Rev Endocrinol Metab Disorders **1:**109–121.
16. Kim PS, Kwon OY, Arvan P 1996 An endoplasmic reticulum storage diseases causing congenital goiter with hypothyroidism. J Cell Biol **133:**517–527.
17. Medeiros-Neto G, Kim PS, Yoo SE, Vono J, Targovnik HM, Camargo R, Hossain SA, Arvan P 1996 Congenital hypothyroid goiter with deficient thyroglobulin. Identification of an endoplasmic reticulum storage disease with induction of molecular chaperones. J Clin Invest **98:**2838–2844.
18. Lamas L, Anderson PC, Fox JW, Dunn JT 1989 Consensus sequences for early iodination and hormonogenesis in human thyroglobulin. J Biol Chem **264:**13541–13545.
19. Vono J, Lima N, Knobel M, Medeiros-Neto G 1996 The effect of oral administration of iodine to patients with goiter and hypothyroidism due to defective synthesis of thyroglobulin. Thyroid **6:**11–15.
20. van Voorthuizen WF, de Vijlder JJM, van Dijk JE, Tegelaers WHH 1978 Euthyroidism via iodine supplementation in hereditary congenital goiter in goats with thyroglobulin deficiency. Endocrinology **103:**2105–2111.

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2. Mariela Caputo, Carina M. Rivolta, Sebastían A. Esperante, Laura Gruñeiro-Papendieck, Ana Chiesa, Claudia G. Pellizas, Rogelio González-Sarmiento, Héctor M. Targovnik. 2007. Congenital hypothyroidism with goitre caused by new mutations in the thyroglobulin gene. *Clinical Endocrinology* **67**:3, 351-357. [CrossRef]
3. Jussara Vono-Toniolo , Carina M. Rivolta , Héctor M. Targovnik , Geraldo Medeiros-Neto , Peter Kopp . 2005. Naturally Occurring Mutations in the Thyroglobulin GeneNaturally Occurring Mutations in the Thyroglobulin Gene. *Thyroid* **15**:9, 1021-1033. [Abstract] [PDF] [PDF Plus]
4. Fernando M Mendive, Carina M Rivolta, Rogelio Gonz??lez-Sarmiento, Geraldo Medeiros-Neto, H??ctor M Targovnik. 2005. Nonsense-Associated Alternative Splicing of the Human Thyroglobulin Gene. *Molecular Diagnosis* **9**:3, 143-149. [CrossRef]
5. Meyer Knobel , Geraldo Medeiros-Neto . 2003. An Outline of Inherited Disorders of the Thyroid Hormone Generating SystemAn Outline of Inherited Disorders of the Thyroid Hormone Generating System. *Thyroid* **13**:8, 771-801. [Abstract] [PDF] [PDF Plus]
6. Christian M. Moya , Viviana Varela , Carina M. Rivolta , Fernando M. Mendive , Héctor M. Targovnik . 2003. Identification and Characterization of a Novel Large Insertion/Deletion Polymorphism of 1464 Base Pair in the Human Thyroglobulin GeneIdentification and Characterization of a Novel Large Insertion/Deletion Polymorphism of 1464 Base Pair in the Human Thyroglobulin Gene. *Thyroid* **13**:4, 319-323. [Abstract] [PDF] [PDF Plus]
7. Carina M. Rivolta , Christian M. Moya , Fernando M. Mendive , Héctor M. Targovnik . 2002. Genotyping and Characterization of Two Polymorphic Microsatellite Markers Located Within Introns 29 and 30 of the Human Thyroglobulin GeneGenotyping and Characterization of Two Polymorphic Microsatellite Markers Located Within Introns 29 and 30 of the Human Thyroglobulin Gene. *Thyroid* **12**:9, 773-779. [Abstract] [PDF] [PDF Plus]