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New insights into thyroglobulin gene: Molecular analysis of seven novel mutations associated with goiter and hypothyroidism

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ABSTRACT

The thyroglobulin (TG) gene is organized in 48 exons, spanning over 270 kb on human chromosome 8q24. Up to now, 62 inactivating mutations in the TG gene have been identified in patients with congenital goiter and endemic or non-endemic simple goiter.

The purpose of the present study was to identify and characterize new mutations in the TG gene. We report 13 patients from seven unrelated families with goiter, hypothyroidism and low levels of serum TG. All patients underwent clinical, biochemical and imaging evaluation. Single-strand conformation polymorphism (SSCP) analysis, endonuclease restriction analysis, sequencing of DNA, genotyping, population screening, and bioinformatics studies were performed.

Molecular analyses revealed seven novel inactivating TG mutations: c.378C>A [p.Y107X], c.2359C>T [p.R768X], c.2736delG [p.R893fsX946], c.3842G>A [p.C1262Y], c.5466delA [p.K1803fsX1833], c.6000C>G [p.C1981W] and c.6605C>G [p.P2183R] and three previously reported mutations: c.886C>T [p.R277X], c.6701C>A [p.A2215D] and c.7006C>T [p.R2317X]. Six patients from two families were homozygous for p.R277X mutation, four were compound heterozygous mutations (p.Y107X/p.C1262Y, p.R893fsX946/p.A2215D, p.K1803fsX1832/p.R2317X), one carried three identified mutations (p.R277X/p.C1981W-p.P2183R) together with a hypothetical micro deletion and the remaining two siblings from another family with typical phenotype had a single p.R768X mutated allele.

In conclusion, our results confirm the genetic heterogeneity of TG defects and the pathophysiological importance of altered TG folding as a consequency of truncated TG proteins and missense mutations located in ACHE-like domain or that replace cysteine.

1. Introduction

Thyroid dyshormonogenesis due to thyroglobulin (TG) gene mutations have an estimated incidence of approximately 1 in 100,000 newborns (Targovnik et al., 2010a, 2011). The clinical spectrum ranges from euthyroid to mild or severe hypothyroidism. The majority of patients have congenital goiter or goiter appearing shortly after birth (Targovnik et al., 2010a, 2011).

Human TG gene is a single copy gene, 270 kb long which maps on chromosome 8q24 and contains an 8.5-kb coding sequence divided into 48 exons (Targovnik et al., 2010a, 2011). Transcription of TG is highly specific to the thyroid cells and is under control of the coordinated action of a master set of transcription factors that includes the homeodomain protein NKX2.1 (TTF-1), the forkhead-domain protein FOXE1 (TTF-2) and the paired-domain protein PAX8 (Targovnik et al., 2010a, 2011). However, a recently report showed that some chondrocytes have the ability to express TG (Endo and Kobayashi, 2011).

TG is a large homodimeric secretory protein with a high degree of glycosylation. The preprotein monomer is composed of a leader peptide of 19 amino acids followed by 2748-amino-acid

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polypeptide (van de Graaf et al., 2001; Targovnik, 2012). Its main function is to provide the macromolecular precursor for synthesis and storage of thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). It is also an important storage of iodine when external odine availability is limited (Targovnik, 2012). Eighty percent of the overall TG monomer encloses three regions with repeat domains (van de Graaf et al., 2001; Lee et al., 2011; Targovnik, 2012). Region I comprises 10 of the 11 TG type-1 repeats, a linker and hinge segments. Region II contains 3 TG type-2 repeats and the 11th TG type-1 repeat, whereas region III contains five TG type-3 repeats. The remaining 20%, that constitutes the carboxy-terminal domain of the molecule, is not repetitive and shows significant homology with the acetylcholinesterase (ACHE-like domain) (van de Graaf et al., 2001; Lee et al., 2011; Targovnik, 2012).

Sixty-two mutations have been identified and characterized in the human TG: 14 splice site mutations, 12 nonsense mutations, 25 missense mutations, eight deletions (six single and two involving a large number of nucleotides) and three single nucleotide insertions (Ieiri et al., 1991; Corral et al., 1993; Targovnik et al., 1993, 1995, 2001, 2010b, 2012; Pérez-Centeno et al., 1996; Hishinuma et al., 1999, 2005, 2006; van de Graaf et al., 1999; González-Sarmiento et al., 2001; Caron et al., 2003; Gutnisky et al., 2004; Rivolta et al., 2005; Alzahrani et al., 2006; Kitanaka et al., 2006; Caputo et al., 2007a,b; Kanou et al., 2007; Kim et al., 2008; Pardo et al., 2008, 2009; Niu et al., 2009; Machiavelli et al., 2010; Peteiro-Gonzalez et al., 2010; Raef et al., 2010; Citterio et al., 2011; Narumi et al., 2011; Moya et al., 2011; Kahara et al., 2012; Liu et al., 2012). Because TG mutations are inherited in an autosomal recessive manner, the patients should be homozygous or compound heterozygous for gene mutations and the parents should be carriers of one TG mutation.

In the present study we report 13 patients from seven unrelated families with goiter, hypothyroidism, and low levels of serum TG. Analysis of the TG gene revealed seven novel mutations: p.Y107X, p.R768X, p.R893fsX946, p.C1262Y, p.K1803fsX1832, p.C1981W, and p.P2183R, and three previously reported mutations: p.R277X (van de Graaf et al., 1999; Gutnisky et al., 2004; Rivolta et al., 2005; Caputo et al., 2007a,b; Pardo et al., 2009; Machiavelli et al., 2010; Peteiro-Gonzalez et al., 2010; Citterio et al., 2011), p.A2215D (Caputo et al., 2007a; Pardo et al., 2008, 2009; Machiavelli et al., 2010), and p.R2317X (Machiavelli et al., 2010; Liu et al., 2012). Six patients from two families were homozygous for p.R277X mutation, four were compound heterozygous mutations (p.Y107X/p.C1262Y, p.R893fsX946/p.A2215D, p.K1803fsX1832/p.R2317X), one carried three identified mutations (p.R277X/p.C1981W-p.P2183R) together with a hypothetical micro deletion and the remaining two siblings from another family with typical phenotype had a single p.R768X mutated allele.

2. Materials and methods

2.1. Patients

Patients selected to participate in this study had goiter, hypothyroidism, elevated serum TSH, low serum total T_4 levels with simultaneous low or normal serum T_3 levels, low serum TG concentration and negative anti-TG and anti-TPO antibodies. Laboratory testing is shown in Table 1 and Fig. 1. All the patients came from iodide-sufficient areas. Families A (III-1, III-2 and III-3), B (II-1, II-2 and II-3), C (II-4), D (II-3 and II-4) and E (II-1) were followed at Endocrine Unit of 'Hospital de Niños Santísima Trinidad', family F (II-2) was followed at Endocrine Division of 'Hospital de Niños Ricardo Gutiérrez', and family G (II-1 and II-2) was followed at Endocrinology Department of Queen Alexandra Hospital. The family pedigrees are shown in Fig. 1.

Therapy with L-T_4 was initiated at doses shown in Table 1 and adjusted according to weight and TSH levels during the years following at diagnosis. In all patients the goiter remained after long-term substitution therapy. However, 6 of them detected by newborn screening (II-4 of family C, II-3 and II-4 of family D, II-1 of family E, and II-1 of family G) or fetal ultrasound (II-2 of family G) did not show development of large goiters, confirming the beneficial effects of early treatment.

Written informed consent was obtained from the parents of the children involved in this study: the research project was approved by the institutional review board.

2.1.1. Family A

2.1.1.1. Patient III-1. The patient is the first child of an unrelated couple. He was referred to the endocrinnology centre at the age of 15.8 due to short stature, goiter, clinical signs of hypothyroidism and mental retardation. At the age of 7, he was diagnosed with hypothyroidism in another institution and received replacement therapy in an interrupted manner. He was not evaluated through the neonatal screening program because the test was not still widely implemented at the time of his birth. At present, he attends a special school.

2.1.1.2. Patient III-2. This patient is III-1's brother diagnosed at 18 days of life, through the neonatal screening program. Since then, he was treated with L-T_4 replacement therapy irregularly for 3 years; parents decided to stop therapy between 3 and 5 years of age. He was referred to the endocrinology center at the age of 5.9 due to short stature, swollen facies, pale skin, goiter and mental retardation. He attends primary school and has learning difficulties, he receives psychopedagogical assistance.

2.1.1.3. Patient III-3. He is patient III-1's younger brother. His congenital hypothyroidism was detected at 42 days of life. He was born at term after a noncomplicated pregnancy and delivery. Clinical examination showed goiter, jaundice and clamping of the umbilical cord at 13 days. He grew up without developmental disturbance or intellectual impairment.

2.1.2. Family B

2.1.2.1. Patient II-1. She is the first child of a non-consanguineous couple referred from the rural area at 16 years of age due to short stature, goiter and mental retardation. Ultrasound showed an enlargement of the gland (Table 1) with heterogeneous pattern of micronodular appearance. In the lower region of left thyroid lobe there is a nodule image of 28 × 9 mm.

2.1.2.2. Patient II-2. This patient is II-1's sister diagnosed with congenital hypothyroidism at the age of 14. At presentation, she showed short stature, convergent strabismus, swollen facies, dry and cold skin, swollen abdomen and a very large asymmetrical goiter, with predominance of the left lobe and of soft consistency. Thyroid ultrasound showed a big enlargement of the gland (Table 1) with a heterogeneous pattern. She had severe psycho-intellectual retardation and did not finish primary school.

2.1.2.3. Patient II-3. Patient II-3, patient II-1's sister, was referred at 12 years of age due to short stature, signs and symptoms of hypothyroidism and a very large asymmetrical goiter, with increasing the consistency. Thyroid ultrasound showed an enlarged gland (Table 1) with inhomogeneous pattern. Right thyroid lobe was dominated by a solid macronodule. Left thyroid lobe showed macro and micronodular patterns all along, the dominant one being of 4 mm. No adenopathies were observed. Fine-needle aspiration biopsy showed a hyperplastic nodular goiter associated with thyroiditis.

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