Thyroid hormone resistance: a novel mutation in thyroid hormone receptor beta (THRB) gene - case report

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Thyroid hormone resistance (THR) is a dominantly inherited syndrome characterized by reduced sensitivity to thyroid hormones. It is usually caused by mutations in the thyroid hormone receptor beta (THRB) gene. In the present report, we describe the clinical and laboratory characteristics and genetic analysis of patients with a novel THRB gene mutation.

The index patient had been misdiagnosed as hyperthyroidism and treated with antithyroid drugs since eight days of age. Thyroid hormone results showed that thyrotropin (thyroid-stimulating hormone, TSH) was never suppressed despite elevated thyroid hormone levels, and there was no symptom suggesting hyperthyroidism. A heterozygous mutation at codon 350 located in exon 9 of the THRB gene was detected in all the affected members of the family.

It is important to consider thyroid hormone levels in association with TSH levels to prevent inappropriate treatment and the potential complications, such as clinical hypothyroidism or an increase in goiter size.

Key words: thyroid hormone resistance, thyroid hormone receptor beta gene (THRB), thyrotropin.

Thyroid hormone resistance (THR) is a dominantly inherited syndrome of reduced target tissue responsiveness to thyroid hormones[1]. The syndrome is characterized by inappropriately normal or even elevated thyroid-stimulating hormone (TSH, thyrotropin) levels associated with elevated serum levels of free triiodothyronine (FT_3) and free thyroxine (FT_4). In 85% of the cases, THR is caused by a mutation in the thyroid hormone receptor beta (THRB) gene coding for thyroid hormone receptor beta unit. The mutant receptor exerts a dominant negative effect over the normal one, resulting in a functional impairment[2].

The clinical presentation and severity of hormone resistance are highly variable. Although the majority of patients are clinically euthyroid, some patients display signs and symptoms of hyperthyroidism, and rarely of hypothyroidism[3]. Even in the same individual, the manifestations of THR may differ from one tissue to another, reflecting the variable degree of tissue responsiveness to thyroid hormones. In addition, clinical changes over time are frequent, and affected subjects within the same family can show different manifestations of the disease[2,4,7]. The relative levels of the mutant and normal receptors, the difference in distribution of thyroid hormone receptor isoforms in different tissues, as well as the genetic heterogeneity of cofactors might explain the variability of tissue responsiveness to thyroid hormones in THR.

Goiter is the most common clinical finding, reported in 65-95% of cases[3]. Other symptoms and signs found at diagnosis include sinus tachycardia, psychological abnormalities, developmental delay, short stature, delayed bone age, hearing loss, and recurrent ear, nose and throat infections[3,8]. When tachycardia is the presenting symptom (33-75%), or if elevated FT_4 levels are discovered at birth,

Table I. Thyroid function tests and treatment history before referral to our hospital

Age	TSH (mIU/L)	TT_4 (μg/dl)	FT_4 (pmol/L)	Treatment
8 days	28.2	28	59.4	PTU
1.4 months	61.5	4.1	9	Na L-thyroxine initiated PTU discontinued
3 months	5.43	24	59.7	No treatment
4 months	4.3	21.7	54	MTZ
5 months	10.6		57.5	MTZ
6 months	8.21		32.3	MTZ
8 months	8.73		29.4	MTZ
Normal Range	0.3-5	4.5-11	12-22	

TSH: Thyrotropin/thyroid-stimulating hormone. TT_4: Total thyroxine. FT_4: Free thyroxine. MTZ: Methimazole. PTU: Propylthiouracil.

Table II. Symptoms and Clinical Information about the Index Case and Family Members

	III.4 (Brother)	III.5 (Index case)	III.6 (Cousin 1)	III.7 (Cousin 2)	II.2 (Mother)	II.4 (Aunt)
Sex	M	M	M	M	F	F
Age at diagnosis	6 years	10 months	4 years, 8 months	13 months	25 years	27 years
Reason for investigation	Screening	Family history	Screening	Screening	Screening	Screening
Birthweight (g)	2600	3300	2900	2800	NA	NA
Gestational week	32 weeks	Term	Term	Term	NA	NA
Height SDS	-0.89	0.11	0.22	-0.11	NA	NA
Weight (Percentile)	10	25-50	25-50	25	NA	NA
Tachycardia	—	Sinus tachycardia (newborn period)	—	—	—	—
Goiter	—	—	—	—	—	—
Hearing defect	—	—	—	—	—	—
Previous treatment	—	Yes PTU, MTZ	—	—	Yes PTU, SSRI	—
Thyroid USG	Slightly non-homogeneous	Normal	Normal	NA	Thyroid nodule (1x0.7 cm)	NA

PTU: Propylthiouracil. MTZ: Methimazole. SSRI: Selective serotonin reuptake inhibitor. THR: Thyroid hormone resistance. USG: Ultrasonography. NA: Not available.

this may lead to an erroneous diagnosis of hyperthyroidism, unless measurable TSH levels are recognized. THR is caused by a defective thyroid hormone receptor (TR)-beta receptor pathway, associated with a normal α receptor function. This may lead to signs of thyrotoxicosis in tissues with a predominant TR-alpha expression, such as the heart, in which elevated T4 and T3 may cause tachycardia[9,10]. The differential diagnosis between THR and hyperthyroidism is mandatory, in order to prevent an inappropriate administration of

Table III. Thyroid Function Tests and Thyroid Antibody Status of the Index Case and Family Members

	III.4	III.5	III.6	III.7	II.2	II.4	Normal ange
TSH mIU/L	3.22	13.28	4.9	5.33	3.32	3.35	0.27-4.2
FT_3 pmol/L	19.81	9.97	20.48	12.73	13.37	9	3.1-6.8
FT_4 pmol/L	44.1	37.3	57.14	40.94	40.04	29.82	12-22
Anti-TPO Ab IU/mL	10	8.8	10	11	389	NA	0-60
Anti-TG Ab IU/mL	<20	<20.0	<20	<20	261	NA	0-40
TSH-R Ab U/L	<1	<1	<1	<1	<1	NA	0-1

TSH: Thyrotropin/thyroid-stimulating hormone. FT3: Free triiodothyronine. FT4: Free thyroxine. Anti-TPO Ab: Antithyroid peroxidase antibody. Anti-TG Ab: Antithyroglobulin antibody. TSH-R Ab: Thyrotropin receptor antibody. NA: Not available.

antithyroid drugs. Therefore, thyroid hormones (FT_4, FT_3) should be evaluated together with TSH levels for a proper diagnosis. In the present work, we report the clinical findings of a family, in which THR was caused by a novel mutation of the THRB gene (S350L).

Case Report

The index case was a 15-month-old male child, referred to the pediatric endocrinology clinic at the age of 10 months, with a presumptive diagnosis of neonatal hyperthyroidism. He was the second child born to non-consanguineous Turkish parents, after a full-term pregnancy and an uneventful delivery, with a birthweight of 3300 g.

On the eighth day after birth, thyroid function tests were performed in another hospital, since his mother had been diagnosed with hyperthyroidism during pregnancy. Elevated total T_4, free T_4 and TSH levels were found; in addition, he had tachycardia, weight loss, irritability, and increased stool frequency, and consequently, was started on antithyroid medication (propylthiouracil [PTU] 5 mg/kg/d) with a diagnosis of neonatal hyperthyroidism. Clinical information and follow-up data from birth to 10 months are shown in Table I.

At the time of referral to our clinic (10 months old), his weight was 9.4 kg (50-75 percentile), length 73 cm (50 percentile), and head circumference 46 cm (90 percentile); his neurological development was appropriate for his age, and the resting heart rate was 110 beats/minute. Thyroid function tests showed elevated TSH despite high levels of FT_4 and FT_3, suggestive of THR, and consequently, the antithyroid medications were discontinued. Thyroid antibody screening (antithyroid peroxidase antibody [TPO-Ab], antithyroglobulin antibody [Tg-Ab], and thyrotropin receptor antibody [TRAb]) were negative, and thyroid ultrasonography revealed a normal thyroid gland volume with homogeneous echo pattern. On 24-hour Holter monitoring, a normal sinus rhythm with sinus tachycardia (mean heart rate 130 beats/minute) was found, and the echocardiogram was normal. During the follow-up since 10 months of age, the patient remained clinically euthyroid, without goiter or tachycardia. Audiogram and growth rate were normal as well as the cognitive and behavioral functions, as assessed by Denver Developmental Screening Test.

The biochemical evaluation of first-degree

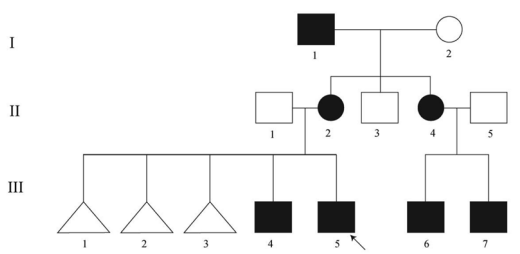

Fig. 1. Pedigree of the family
III.1, III.2, III.3 Triangles represent previous abortions.

Table IV. Alignment of S350L

Homo sapiens	339	GQLKNGGLGVVSDAIFDLGMS	359
Ailuropoda melanoleuca	345	GQLKNGGLGVVSDAIFDLGMS	365
Bos taurus	206	GQLKNGGLGVVSDAIFDLGMS	226
Bos taurus	398	GQLKNGGLGVVSDAIFDLGMS	418
Callithrix jacchus	339	GQLKNGGLGVVSDAIFDLGMS	359
Canis lupus familiaris	354	GQLKNGGLGVVSDAIFDLGMS	374
Danio rerio	264	GQLKNGGLGVVSDAIFDLGVS	284
Equus caballus	339	GQLKNGGLGVVSDAIFDLGMS	359
Gallus gallus	247	GQLKNGGLGVVSDAIFDLGMS	267
Macaca mulatta	339	GQLKNGGLGVVSDAIFDLGMS	359
Monodelphis domestica	354	GQLKNGGLGVVSDAIFDLGMS	374
Mus musculus	339	GQLKNGGLGVVSDAIFDLGMS	359
Nomascus leucogenys	339	GQLKNGGLGVVSDAIFDLGMS	359
Oryctolagus cuniculus	354	GQLKNGGLGVVSDAIFDLGMS	374
Oryzias latipes	256	DQLKNGGLGVVSDAIFDLGVS	276
Pan troglodytes	339	GQLKNGGLGVVSDAIFDLGMS	359
Rattus norvegicus	375	GQLKNGGLGVVSDAIFDLGMS	395
Salmo salar	274	GQLKNGGLGVVSDAIFDLGLS	294
Xenopus tropicalis	261	GQLKNGGLGVVSDAIFDLGVS	281

relatives of the index case revealed other affected family members, including the mother (II.2), brother (III.4), aunt (II.4), and two cousins (III.6 and III.7). The brother of the index patient (III.4) had attention deficiency hyperactivity disorder (ADHD), as described in some THR individuals during childhood[11]. The first-degree relatives were totally asymptomatic (cases II.4, III.6, and III.7) and were detected during the family screening, while case II.4 was identified during routine testing performed during pregnancy. None of the family members had goiter.

The pedigree of this family is shown in Figure 1, while the clinical and laboratory findings are shown in Tables II and III, respectively.

Genomic DNA Analysis

Informed consent for the molecular studies was retrieved from all members or their representatives. DNA was extracted from whole blood using standard methods[12]. All coding exons (from 3 to 10) of the THRB gene were amplified using intronic oligonucleotide primers, as previously described[12]. Polymerase chain reaction (PCR) products were directly sequenced. In particular, an aliquot of 3-10 ng/100 bp of purified DNA and 3.2 pmol of either the forward or reverse primer were used in standard cycle sequencing reactions with ABI PRISM Big Dye terminators and run on an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, CA, USA). Both exonic sequences and intronic boundaries were evaluated. We identified a heterozygous mutation at codon 350 (nucleotide c.1049 TTA→ TCA), located in exon 9 of the THRB gene, which resulted in replacement of the normal leucine with a serine (S350L) in all the affected members of the family (II.2, II.4, III.1, III.2, III.3, III.4).

This is the first time that the mutation S350L is being reported in a family with THR (Fig. 2). Alignment of S350L is shown in Table IV.

Discussion

More than 2,000 patients with THR have been reported, and in 85-90% of cases, a point mutation or small deletions in the THRB, coding for TR-β, are found[13].

T_3 is the biologically active thyroid hormone and its actions are mediated by nuclear receptors (TRs), which can bind T_3 with high affinity.

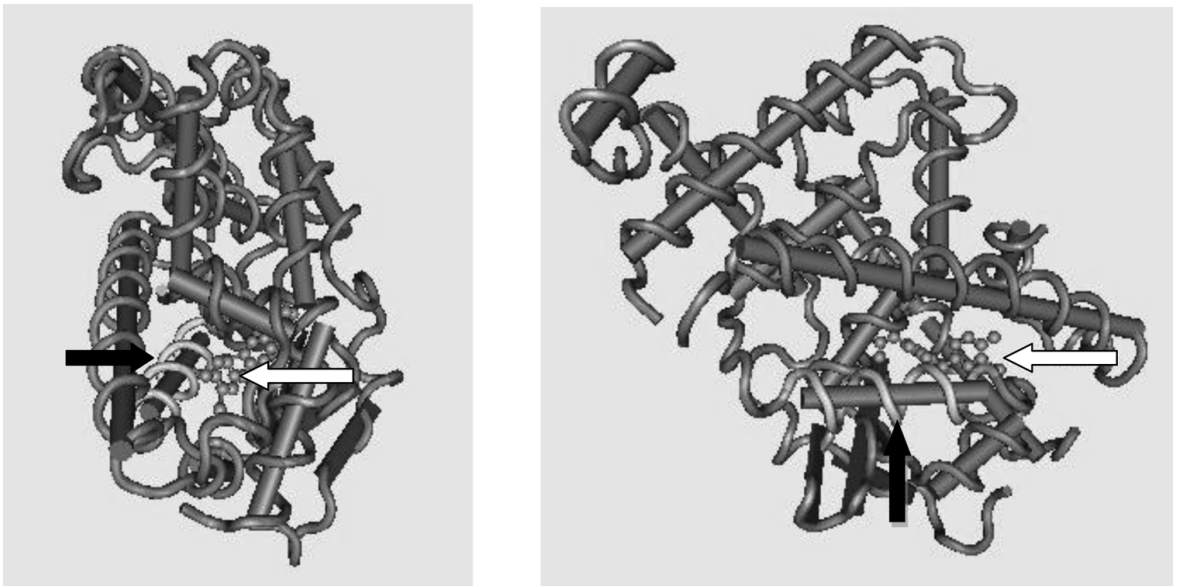

Fig. 2. Crystal structure of TR-beta bound to T3 (black arrow) showing the position of helix 349-360 (white arrow).

This hormone-receptor interaction activates or represses specific target genes[13]. All the described mutations in TR-β cause a reduced binding affinity for the ligand (T_3) or an impaired interaction with cofactors. As a result, the mutant TR interferes with the function of normal TRs (dominant negative effect), which explains the dominant mode of inheritance of this syndrome. The present case report describes a family with a new mutation in the THRB gene (S350L).

The majority of TR-β mutations are located within two "hot spot regions" separated by a highly conserved region of 80 amino acids, spanning from codon 349 to 429, previously described as a "cold region"[12,14]. However, over the years, new mutations have been identified in this area, extending the boundary of the hot spot regions from codons 309 to 353 and from 374 to 461, respectively. A third cluster of mutations has been identified within the hinge region (codon 234-282)[15]. In the present study, the substitution at codon 350 of the polar serine with a non-polar leucine, characterized by a higher molecular weight, is predicted to damage the TR function. This is supported by the results of functional studies on previously reported mutations, affecting amino acids close to S350. As an example, the V349M mutation shows a significant dominant negative activity in vitro, greater as TR-β2 vs TR-β1 isoforms[16]. Moreover, the artificial mutation A352T displays a reduced T_3 binding as well as a reduced response to T_3 in transient transactivation experiments[14]. On the basis of the crystal structure of helix 349-360 of ligand binding domain (LBD), we suggest that S350R might display features similar to these latter mutations.

The clinical presentation of THR is highly heterogeneous and depends on the degree of compensation achieved at the peripheral tissue level by the high concentrations of circulating free thyroid hormones. The efficacy of this compensation mechanism may vary among individuals, in different tissues of the same subject, and even in different periods of an individual's life. In addition, family members harboring the same mutation can display different clinical features[17]. The presence of a TSH-secreting pituitary adenoma should be ruled out when clinical investigations are not conclusive; however, the presence of other affected family members strongly suggests the diagnosis of THR, since no case of familial TSH-secreting pituitary tumor has been described so far, except for patients affected with type 1 multiple endocrine neoplasia (MEN 1).

There is no treatment available to correct the defect causing THR3. Treatment is not required in most patients, as elevated thyroid hormones compensate for the partial tissue resistance,

and consequently, the majority of the patients are clinically euthyroid. In the present study, all the affected family members of our patient were clinically euthyroid and were not started on any treatment. When resting tachycardia is the most significant symptom, a cardioselective beta-blocker is often given. Atenolol seems to be the best choice, because it does not inhibit peripheral T_4 to T_3 conversion.

Other treatment options include TRIAC and DT_4 (dextrothyroxine) for patients presenting with thyrotoxic symptoms, or supraphysiological doses of levothyroxine (LT_4) for patients with clinical features of hypothyroidism[17]. On the contrary, any attempt to reduce the elevated thyroid hormone concentrations should be avoided, since it may lead to hypometabolic signs and symptoms, especially in the first months of life, when thyroid hormones are essential in neurological development. In addition, an increase in TSH levels, produced by anti-thyroid drugs, is often followed by an increase in goiter size and a possible pituitary hyperplasia.

In conclusion, a new mutation in the THRB gene is described herein in a family with THR. The affected members of the family are clinically euthyroid, and no treatment was necessary. However, some members of the family were administered antithyroid medication prior to an appropriate diagnosis. This should be avoided, since inappropriate antithyroid therapy may lead to clinical hypothyroidism as well as increased goiter size.

Finally, during the newborn period, thyroid hormone levels should be evaluated together with TSH levels for a proper diagnosis of THR, since many patients do not need any treatment other than follow-up.

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