

A Novel Mutation (M310L) in the Thyroid Hormone Receptor β Causing Resistance to Thyroid Hormone in a Brazilian Kindred and a Neonate

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Resistance to thyroid hormone (RTH) is an inherited syndrome of reduced tissue responsiveness to thyroid hormone (T3) caused by mutations in the thyroid hormone receptor β (TR β). The index patient of the family reported here, a 17-year-old woman, came to medical attention because of a diffuse goiter, short stature, and learning disabilities. Biochemical tests revealed an elevated free T4 of 5.2 ng/dl (0.8–2.1), a T3 of 270 ng/dl (80–220), and a non-suppressed TSH of 1.79 mU/l (0.4–4). Administration of exogenous T4 or T3 did not result in the usual TSH suppression, prompting the clinical diagnosis of RTH. Her father and one of her brothers also had clinical and biochemical findings consistent with RTH. Direct sequence analysis of the TR β gene revealed a heterozygous transition 928A>G in exon 9 resulting in substitution of methionine 310 by leucine (M310L). This novel receptor mutant has a reduced affinity for T3 (~10% of normal) and dominant negative properties that are similar in comparison to other RTH mutations. The index patient had a normal pregnancy and delivery. At birth, the female neonate had no goiter, a significantly elevated T4, and increased TSH. The diagnosis of RTH was confirmed by sequencing the TR β gene. She was underweight at birth and her length was between the 5th and 10th percentile. At 26 months, her height remained at the 10th percentile but her bone age was 18 months, suggesting mild hypothyroidism at the level of the bone. In contrast, increased heart rate and restlessness are consistent with hyperthyroidism in other tissues, such as the heart and possibly the brain. © 2000 Academic Press

Key Words: Thyroid hormone receptor; hormone resistance; mutation; neonatal diagnosis.

Resistance to thyroid hormone (RTH) is characterized by decreased responsiveness to thyroid hormone (TH) (1,2). Biochemically, the syndrome is defined by elevated free thyroid hormones and an inappropriately normal, or elevated, level of thyroid stimulating hormone (TSH). The clinical spectrum is highly variable and ranges from isolated biochemical abnormalities to a constellation of features that includes goiter, variable features of hyper- and hypothyroidism, short stature, delayed bone maturation, and attention deficit hyperactivity disorder (1–3). In one instance, RTH was associated with loss of both thyroid hormone receptor β (TR β) alleles (4), but it is most commonly caused by autosomal dominant mutations in TR β (1,5). More recently, familial cases of RTH without linkage to the TR β locus have been reported, indicating the possibility of nonallelic heterogeneity (6,7).

Most TR β mutations result in reduced binding affinity for T3, although a subset of mutants impairs transcriptional activity despite near normal T3 binding (8,9). Consistent with the dominant mode of transmission, mutant TR β s interfere with the function of the wild type receptor by a dominant negative mechanism (10). On positively regulated genes, the dominant negative effect is thought to occur predominantly through a suppressive complex of the mutant receptor with nuclear corepressors (11). On genes that are negatively regulated by T3, such as the TSH α and β genes, the complex between the mutant TR β and the corepressor activates transcription (11).

The diagnosis of RTH is important to avoid inap-

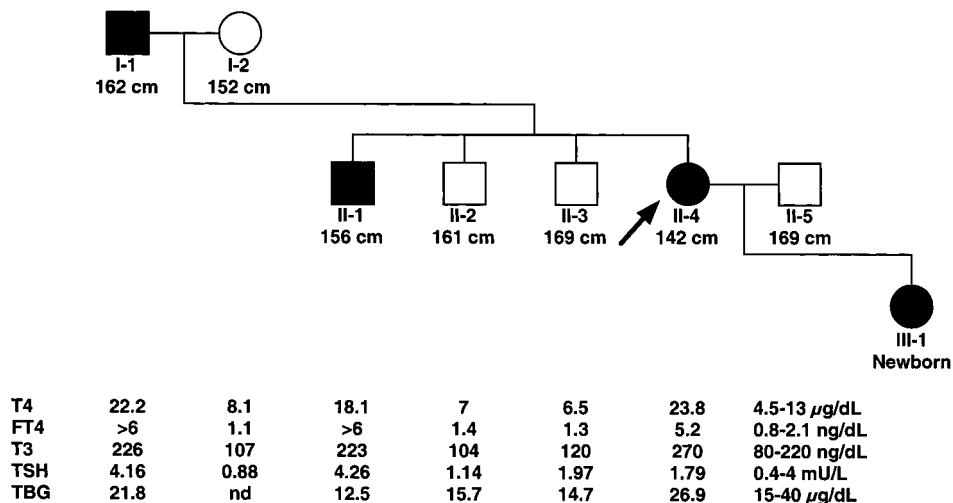


FIG. 1. Pedigree, height, and laboratory findings in a family with the TR β M310L RTH mutation.

appropriate treatment of the condition (1). Although asymptomatic patients do not require intervention, treatment may be required in a subset of patients to ameliorate features of hyper- or hypothyroidism. Because of the importance of T3 for normal development, treatment of thyroid hormone deficiency or excess may be of particular importance in infants, but treatment guidelines are controversial (12–14).

Here we describe the clinical and molecular analysis of a Brazilian family with RTH and its presentation in a newborn.

CASE REPORT AND METHODS

Case Report

A 17-year-old Brazilian woman of Caucasian heritage was evaluated for nervousness, restlessness, and inability to concentrate. Her height and weight were normal at birth, and, with the exception of delayed walking until 2 years of age, her somatic development was normal. Menarche oc-

curred at age 13 and she had regular menses thereafter. She was not aware of any thyroid or other endocrine disorders in her family. On physical examination, her height was 142 cm (<3rd percentile) and weight was 35 kg (<3rd percentile). She had a soft diffuse goiter of about 50 g. Thyroid function tests revealed a normal TSH of 1.79 mU/L (0.4–4), a total T4 of 23.8 $\mu\text{g/dl}$ (4.5–13), a free T4 of 5.2 ng/dl (0.8–2.1), and a T3 of 270 ng/dl (80–220) (Fig. 1, Table 1). Her thyroxine binding globulin was normal with 26.9 $\mu\text{g/dl}$ (15–40). Anti-microsomal antibodies were negative.

Basal TSH, TRH-stimulated TSH after injection of 200 μg TRH iv, and T3 levels were measured after treatment with either levothyroxine (1.43 $\mu\text{g/kg/day}$ for 6 weeks) or liothyronine (5.7 $\mu\text{g/kg/day}$ for 3 and 7 days). Despite significantly elevated T3 levels of more than 600 ng/dl, the TSH was only modestly suppressed to levels of 0.14 mU/l.

After establishing the clinical diagnosis of RTH, the parents and the three brothers of the index

TABLE 1
Thyroid Function Tests of the Index Patient in Response to Exogenous Thyroid Hormone

	Basal	T4 50 μg 6 weeks	T3 200 μg 3 days	T3 200 μg 7 days	Normal range
T3	270	213	590	>600	80–220 ng/dl
TSH 0 min	2.6	1.3	0.14	0.14	0.4–4.0 mU/L
TSH 30 min*	24.3	17.8	4.8	0.93	
TSH 60 min*	18.2	14.9	2.7	0.67	

* After 200 μg TRH i.v.

TABLE 2
Thyroid Function Tests in an Infant with RTH

	TSH (mU/L)	T4 (μ g/dl)	Free T4 (ng/dl)	T3 (ng/dl)	TBG (ng/dl)
Cord blood	23.4 (1–20)	20.5 (6.6–15)			
1 day		>24 (11–21.5)	2.5 (0.68–2.08)	277 (100–740)	3.6 (0.8–5.2)
7 days	10.2 (1–10)	>24 (8.1–20.1)	>6 (1.84–3)	205 (36–316)	2.97 (0.8–5.2)
8 months	3.04 (0.5–6.5)	>24 (5.9–16.3)		301 (105–245)	

Note. The normal ranges for newborns and infants are taken from Ref. (40) and are indicated in parentheses. TBG, thyroxine binding globulin.

patient were examined as well (Fig. 1). Her father (I-1) and her older brother (II-1), who both had a history of significant learning disability and nervousness, had biochemical tests consistent with RTH (Fig. 1).

At the age of 18, the proposita (II-4) had an uneventful pregnancy. Prenatally, the heart rate of the baby varied between 140 to 156 beats per minute. After 37 weeks of gestation, the baby girl was delivered with the use of forceps. The Apgar scores of the infant were 5 at 1 min, and 8 at 5 min postpartum. Her heart rate at birth was 148 beats per minute, and she had no signs of respiratory distress. Her length was 46 cm (5–10th percentile), and her weight was 2.25 kg (<5th percentile). There was no apparent enlargement of the baby's thyroid gland.

Thyroid function tests in the cord blood showed a slightly elevated TSH of 23.4 mU/l (1–20), and an elevated T4 of 20.5 μ g/dl (6.6–15) (Table 2). Direct sequence analysis of exon 9 of the TR β gene confirmed the diagnosis of RTH in the baby. T4 levels were persistently elevated during early infancy, and TSH levels normalized (Table 2). Her serum T3 was initially within the normal range but was elevated at 8 months.

Until age 4 months, the infant grew at the 5th percentile. At 8 months of age, her length was below the 3rd percentile (63.5 cm), her weight was 6.75 kg (5–10th percentile), and her head circumference was 42.5 cm (~50th percentile). Her heart rate was near the upper limit of the normal at 152 beats per minute (<160). The thyroid was not enlarged, and she appeared to have a normal psychomotor development. At 26 months of age, her height was at the 10th percentile and her bone age was delayed (18 months). Weight remained at the 3rd percentile. Her heart rate was elevated at 130 beats per minute and she was restless by history and on clinical examination. A Holter monitor revealed an average heart rate of 124 bpm (age

adjusted mean = 110 bpm), confirming mild persistent tachycardia (15).

DNA Sequencing

After obtaining informed consent, DNA was extracted from peripheral leukocytes of all direct family members. Exons 9 and 10 of the TR β gene were amplified by PCR using primers reported elsewhere (16). The amino acid residues of TR β are numbered according to the consensus nomenclature (17).

Plasmids

The mutant human TR β cDNA was prepared by oligonucleotide-directed mutagenesis and verified by DNA sequencing (18). Mutant and wild type TR β receptor cDNAs were expressed in pCMX as described previously (19). The plasmid PAL-TK-Luc contains two copies of a palindromic TRE (AGGT-CATGACCT) upstream of the thymidine kinase promoter beginning at bp –109 (TK109) in the pA3 luciferase vector (20). DR4-SV40-Luc contains four copies of a direct repeat TRE (AGGTCACTtCAG-GTCA) upstream of the simian virus 40 (SV40) promoter in the pGL3 Luc vector (11). The plasmid TSH α Luc contains 846 bp of the 5'-flanking sequence and 44 bp of exon I from the human glycoprotein hormone α subunit gene in pA3-Luc (21).

T3 Binding Assays

Proteins were translated *in vitro* using the TNT-coupled reticulocyte lysate system (Promega, Madison, WI). T3 binding affinity of TR β mutant and wild type receptors was determined using 2 μ l of *in vitro* translation product with 0.07 nM ¹²⁵I-iodine-labeled T3 and increasing amounts of unlabeled T3 (0.039–0.625 nM) at 4°C for 16 h (20,22). Nonspecific binding was determined in the presence of unlabeled ligand in excess. The amount of bound T3 was measured by a filter binding assay (20,23). The results

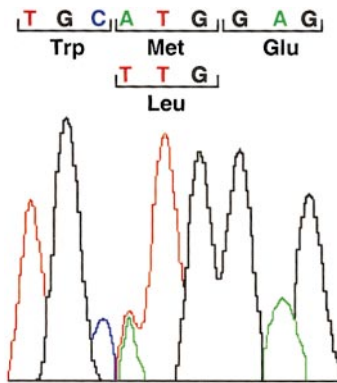


FIG. 2. Nucleotide and amino acid sequence of part of exon 9 encoding the carboxyterminal part of TR β . A heterozygous transition of 928A > T results in the substitution of methionine (ATG) by leucine (TTG) at position 310 (M310L).

represent the mean \pm standard error of six different experiments. Receptor affinity constants (K_a) were calculated using linear regression analysis.

Tissue Culture and Transient-Expression Assays

TSA-201 cells, a clone of human embryonic kidney 293 cells (24), were cultured in Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μ g/mL). Cells were plated in 12-well dishes 24 h before transfection by the calcium phosphate method. Per well, 250 to 500 ng reporter plasmid was cotransfected with 50 to 100 ng of TR β expression plasmids. The total amount of DNA was maintained constant in each reaction by the addition of the empty plasmid without receptor. After an 8-h exposure to the calcium-phosphate-DNA-precipitate, the media was aspirated. Subsequently, DMEM with 10% Dowex resin-stripped fetal bovine serum was added with variable concentrations of T3 as indicated. Cells were harvested after 40 h, and luciferase activity was measured as reported previously (20). All experiments were repeated at least three times. Groups were compared by ANOVA.

RESULTS

Sequence Analysis

All affected patients were heterozygous for a transition of 928A > T in exon 9. This results in the substitution of methionine (ATG) by leucine (TTG) at position 310 (M310L) (Fig. 2).

T3 Binding

T3 binding affinity of the M310L mutant and wild type TR β receptors was determined using *in vitro* translated receptors and a filter binding assay system (20). The wild type receptor displayed a K_a of $12.6 \times 10^9 \text{ m}^{-1}$. In contrast, the affinity of TR β M310L for T3 was significantly reduced by $\sim 90\%$, with a K_a of $1.4 \times 10^9 \text{ m}^{-1}$ ($P < 0.002$) (data not shown).

Transfection Studies

The dominant negative properties of the TR β M310L mutant were examined in transiently transfected cells. Compared to wild type TR β alone, cotransfection of M310L with wild type TR β in a 1:1 ratio significantly reduced the activity of two positively regulated reporter genes in response to T3 in the range of 1.25 to 10 nM (Figs. 3A and 3B). The dominant negative properties of M310L were comparable to G345R, the first reported TR β point mutation associated with RTH (25). In comparison to the RTH mutation F451X, which lacks the last 10 amino acids and the carboxyterminal AF-2 transactivation domain, the dominant negative properties were less pronounced. Using a negatively regulated reporter gene consisting of -846 bp of the human α glycoprotein hormone promoter, cotransfection of M310L inhibited TR β -mediated repression at high doses of T3 (10 nM) (Fig. 4). These *in vitro* findings confirm the anticipated dominant negative properties of the M310L mutant and are consistent with the biochemical findings in the affected individuals (Fig. 1).

DISCUSSION

RTH is most commonly caused by autosomal dominant mutations in TR β (1,2). There is marked allelic heterogeneity and more than 70 TR β gene mutations have been reported to date (5). Hyposensitivity to T3 with an identical phenotype, but without defects in TR β or TR α , is thought to account for about 10% of families with RTH (6,7). As of yet, the gene(s) responsible for RTH in these patients have not been identified.

The proband in this family presented with nervousness and learning difficulties. Abnormal thyroid function tests were detected at age 17 and led to the diagnosis of RTH and the discovery of a novel point mutation in TR β . With a height of 142 cm she is below the 3rd percentile (26), and her height is at

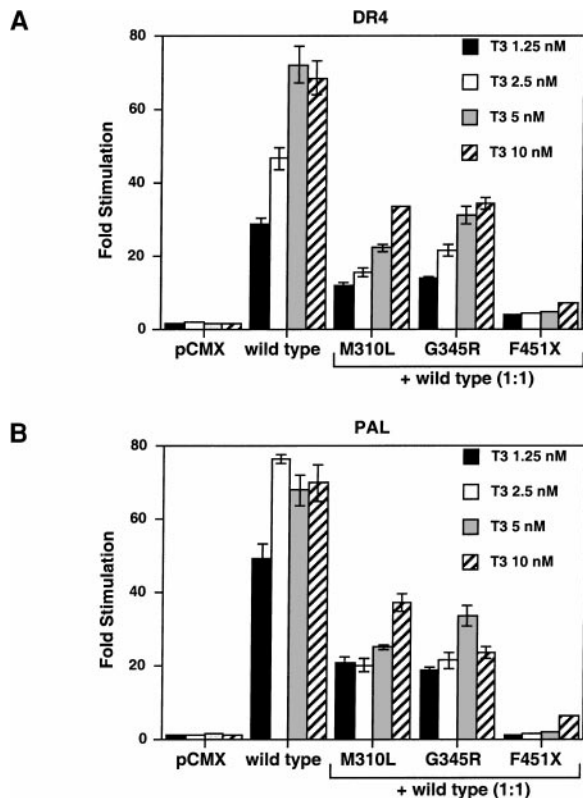


FIG. 3. Functional analysis of TR β mutation M310L on two positively regulated reporter genes. The dominant negative properties of the TR β mutant M310L, G345R, and F451X were determined in transiently transfected TSA cells in the presence of varying doses of T3 ranging from 1.25 to 10 nM. The data are expressed as mean \pm SE of triplicate transfections. (A) DR4-SV40-Luc containing four copies of a direct repeat TRE (250 ng) was cotransfected with empty vector (200 ng), TR β wild type (100 ng) and empty vector (100 ng), or TR β wild type (100 ng) and the TR β mutants (100 ng). (B) PAL-TK-Luc containing two copies of a palindromic TRE (250 ng) was cotransfected with empty vector (100 ng), TR β wild type (50 ng) and empty vector (50 ng), or TR β wild type (50 ng) and the TR β mutants (50 ng).

the lower limit of the parental target height for girls (150.5 ± 8.5). Her father, also affected by RTH, is of normal height (162 cm), the unaffected mother is 152 cm (Fig. 1). The height of her affected brother (II-1) is 156 cm (<3rd percentile). Though he is within the parental target height for boys (163.5 ± 10), he is smaller than his two unaffected brothers who are 161 and 169 cm. It is possible that RTH contributed to short stature in these affected individuals, although the small size of the pedigree and the likelihood that other factors affected height make it difficult to unequivocally establish this relationship.

RTH was suspected in the neonate of the index

patient because of the increase of T4 and TSH in the cord blood (27,28). Clinically, there was no fetal tachycardia, length at birth was normal, weight was below the 3rd percentile, and the baby had no goiter. The diagnosis of RTH was established by demonstrating the TR β mutation in DNA extracted from leukocytes obtained from cord blood. TSH levels were slightly above the upper limit of the normal range after the first week of life, but normalized within the first few months of life (Table 2). Because of the wide range of thyroid function tests during the neonatal period and early infancy, DNA testing is useful to make a definitive diagnosis of RTH in newborns.

The novel TR β mutation causing RTH in this Brazilian family results in substitution of methionine 310 by leucine (M310L). Mutations of methionine 310 have been reported previously in other families with RTH and include substitutions to threonine or isoleucine (29,30). These patients had similar thyroid hormone levels as observed in the patients of this kindred. RTH mutations have been localized to three restricted domains within TR β (1,5). M310L is located in cluster 1, which encompasses helix 6, helix 7, and the connecting loop with helix 8 (31). M310 itself is located in helix 6. Based on the three-dimensional structure of rat TR α , it is thought to make direct contacts with the hormone (31). Thus, M310 is one of several mutations that affect the hydrophobic core which surrounds the ligand.

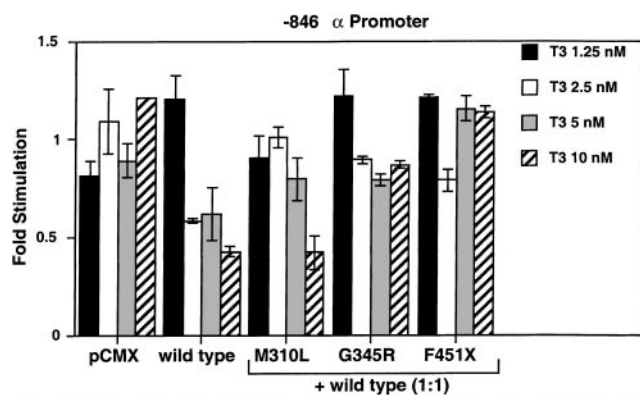


FIG. 4. Functional analysis of TR β mutation M310L on a negatively regulated reporter gene. TSH α -Luc containing 846 bp of the 5'-flanking sequence and 44 bp of exon I from the human glycoprotein hormone α subunit gene in pA3-Luc (250 ng) was cotransfected with empty vector (200 ng), TR β wild type (100 ng) and empty vector (100 ng), or TR β wild type (100 ng) and the TR β mutants (100 ng) in the presence of increasing concentrations of T3. The data are expressed as mean \pm SE of triplicate transfections.

Functionally, the M310L mutation displayed a dominant negative effect that was comparable to the G345R mutation, but less pronounced than F451X, on two positively regulated reporter genes (DR4-4X, pal-2X). F451X lacks the carboxyterminal AF-2 transactivation domain, has negligible T3 binding and transactivation properties, retains normal homodimer and heterodimer formation with RXR, and has pronounced dominant negative and silencing activity (32). The TR β mutant G345R has similar characteristics as F451X, although less pronounced, and it has been shown to form a stable complex with the nuclear corepressor NCoR in the presence of T3 (11). The interaction of TR β mutations with nuclear corepressors correlates with dominant negative potency and appears to provide a molecular explanation for this phenomenon (11,33,34). Consistent with the clinical features, repression of a negatively regulated reporter construct consisting of the human α glycoprotein hormone promoter by M310L was only observed at supraphysiological doses of T3 (Fig. 4).

In the infant girl, TSH levels were slightly above the upper limit of the normal range during the first weeks of life. RTH was confirmed by direct mutational analysis. The initial TSH elevation, presumably reflecting an adaptation of the thyroid axis in postnatal life to the increased demands for thyroid hormone, normalized within the first few months of life (Table 2). Although rare, RTH is an diagnostic consideration when neonatal screening shows mildly increased TSH levels in combination with elevated serum concentrations of thyroxine (Table 2) (27,28).

In the infant affected by RTH, height is in the low normal range, but the bone age suggests mild hypothyroidism at the level of the bone, a relatively common finding in RTH (3). In contrast, hyperthyroidism may be present at the level of the brain and heart. The index patient came to medical attention because of nervousness and inability to concentrate. Restlessness and mild tachycardia are also present in the infant with RTH. Therapeutically, this child may benefit from a treatment with a β -adrenergic blocker, such as atenolol, for the accelerated heart rate. In case of a further delay in bone maturation and a decrease in growth velocity, this treatment may be combined with supraphysiologic doses of levothyroxine (12). Alternatively, the use of triiodoacetic acid could be considered (12,35–39). Currently, there are no definitively established guidelines and therapeutic interventions require particularly careful monitoring (12–14).

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