

Very Severe Resistance to Thyroid Hormone β in One of Three Affected Members of a Family with a Novel Mutation in the *THRB* Gene

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A 13-year-old female with a novel *THRB* gene mutation (c.1033G>T, p.G345C) presented with 3- to 6-fold higher serum iodothyronine levels and more severe clinical manifestation than 2 other family members carrying the same mutation. The leukocytes of the proband expressed both wild-type and mutant *THRB* mRNAs, excluding the possibility of a partial deletion of the allele not carrying the mutation. The proband's fibroblasts showed reduced responsiveness to triiodothyronine compared with those of another affected family member. The more severe clinical and biochemical phenotype suggest a modifier-mediated worsening of the resistance to thyroid hormone.

Keywords: resistance to thyroid hormone, thyroid hormone receptor, mutation, cofactor, non-TR-RTH

Introduction

RESISTANCE TO THYROID HORMONE (RTH) β is a syndrome of reduced tissue responsiveness to thyroid hormone (TH). Most individuals are heterozygous for mutations in the *THRB* gene, which produce receptors that interfere with the function of the normal (wild-type[WT]) receptor. Patients homozygous for a mutant or deleted *THRB* gene have more severe phenotype (1). RTH β without a *THRB* gene mutation referred as non-TR-RTH is clinically and biochemically undistinguishable of heterozygotes with *THRB* gene mutations (2). However, its mechanism remains elusive.

In this study, we describe a family in which one of three affected members harboring a novel mutation in the *THRB* gene manifested more severe clinical and biochemical findings than the other two affected family members.

Subjects

The proband was a 13-year-old female, the first born to nonconsanguineous French Canadian parents. She presented with tachycardia, exercise intolerance, heat intolerance, and tremor. Her history was unremarkable with normal growth, puberty, and regular menses. On physical examination, she had a large goiter, a fine tremor, and nonpitting leg edema (Supplementary Fig. S1). Thyroid

function tests revealed markedly elevated total thyroxine, total triiodothyronine (T3), total reverse triiodothyronine, free thyroxine index, and a slightly high thyrotropin (TSH) (Fig. 1A). Antibodies against the TSH receptor, thyroperoxidase (TPO), and thyroglobulin (TG) were negative. Thyroid ultrasonography showed a large homogenous goiter (Supplementary Fig. S1). Her pituitary magnetic resonance imaging was normal.

The family history included a father with no complaints and a younger brother with exercise intolerance. Both had elevated iodothyronine levels but of distinctly lesser magnitude than those of the proband (Fig. 1A), as well as smaller thyroid glands (Supplementary Table S1). No family member had a heart murmur, and liver or spleen enlargement. Vital signs are summarized in the Supplementary Table S1. The mother had normal thyroid function tests with the exception of a high TG and positive TG and TPO antibodies (Fig. 1A).

Results

The investigation was approved by the Institutional Review Board. Sanger sequencing of the *THRB* gene demonstrated a novel heterozygous missense mutation (c.1033G>T) resulting in the substitution of the normal glycine 345 with a cysteine (p.G345C) in the proband, her father and brother, but

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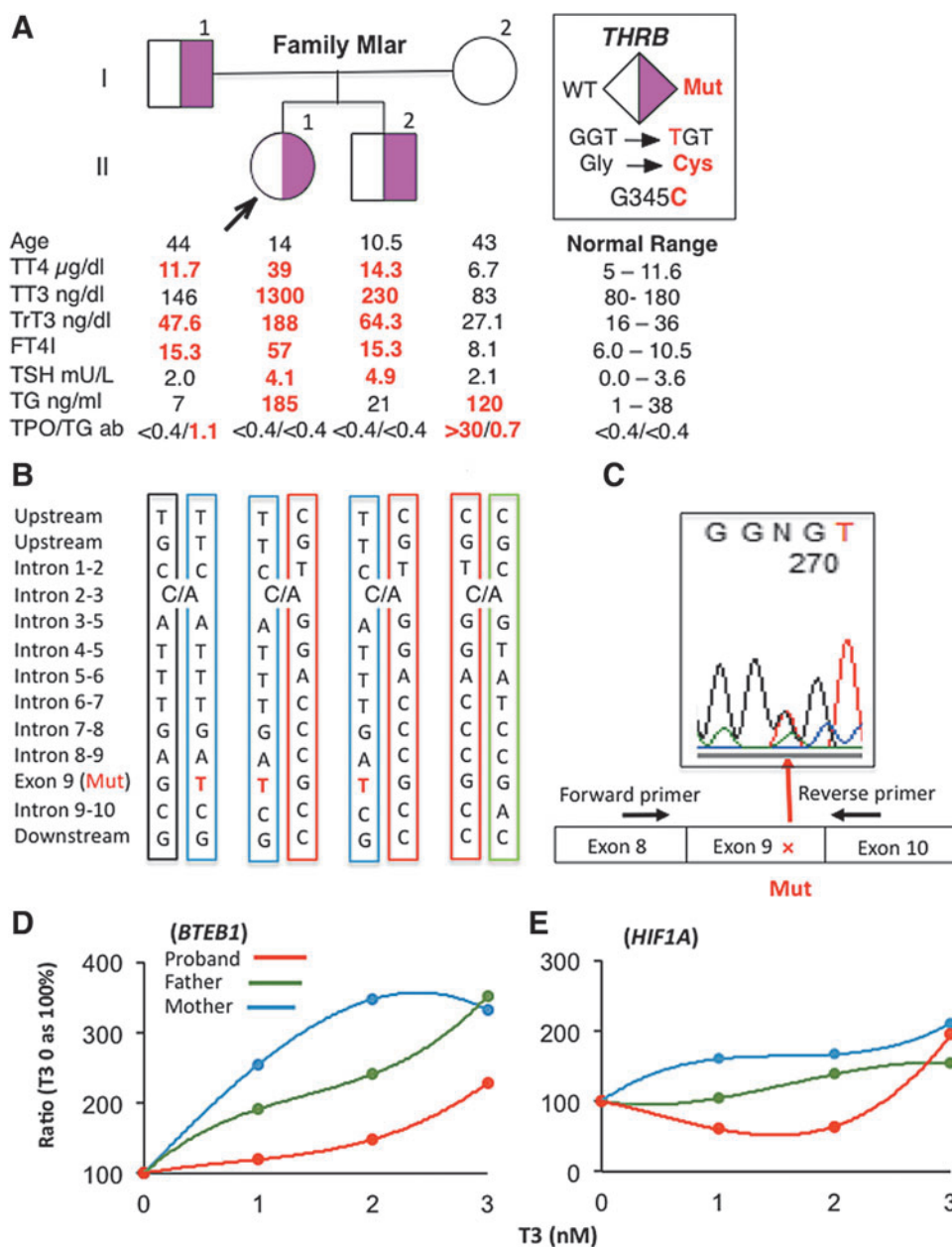


FIG. 1. (A) Pedigree of the family indicating the genotype of each individual and the results of thyroid function tests, aligned with the symbol representing each family member. (B) Results of *THR β* locus haplotyping show that the proband and brother inherited the same maternal allele. (C) Sequence of cDNA derived from white blood cells mRNA of the proband, covering the region of the *THR β* mutation. The location of the primers used in the cDNA amplification is indicated. (D, E) TH responsiveness of the subjects' skin fibroblasts. After culture for 48 hours in medium depleted of TH, fibroblasts were treated with three different amounts of T₃, 0.5, 2, and 5 nM, for additional 24 hours. The relative amount of mRNAs, compared with that in fibroblasts cultured in the absence of T₃, is given as percentage increase. TH, thyroid hormone; T₃, triiodothyronine.

not the mother. No mutations were found in the sequence coding for *THR β 2*. The mother had a WT sequence. The presence of an *albumin* gene gain-of-function mutation to explain the higher iodothyronine levels of the proband was also excluded by sequencing.

As the phenotype of the proband was reminiscent of patients with RTH β expressing only a mutated *THR β* allele (1), the possibility that the proband expresses only the mutant allele through a partial deletion of the maternal allele was considered. Haplotyping revealed that both the proband and her brother shared the same maternal and paternal alleles (Fig. 1B). Furthermore, *THR β* cDNA from the proband's white blood cell, sequenced across exons junctions to prevent amplification of genomic DNA, showed that both the WT and mutant alleles were equally expressed (Fig. 1C), thus excluding the possibility of decreased expression of the normal maternal *THR β* allele.

Finally the tissue sensitivity to TH was assessed *in vitro* using the subjects' cultured skin fibroblasts. The expression of two genes, *BTEB1* and *HIF1A*, positively regulated by TH, showed the largest response to the addition of small increments of T₃ in fibroblasts from the unaffected mother, the smallest response in those of the proband, with an intermediate dose response in fibroblasts from the heterozygous father (Fig. 1D, E). These data confirm the increased severity of RTH β in the proband compared with an affected family member carrying the same heterozygous *THR β* mutation.

Discussion

We report a novel *THR β* gene mutation in an individual with clinical and biochemical manifestations more severe than in the affected sibling and the father harboring the same mutation. The more pronounced resistance to TH was

confirmed in cultured skin fibroblasts. The resistance was not caused by reduced expression of the normal allele inherited from the mother by both the proband and affected brother. While the magnitude of iodothyronine elevation in the proband was comparable to that of homozygotes for *THRB* gene mutations (1), her TSH was only minimally elevated and she showed no delay in growth, bone, and cognitive development, nor hearing loss. An increase in the bioactivity of TSH, previously reported in RTH β (3), cannot explain the large goiter in the proband, a finding that was not present in her affected brother with a similar TSH concentration. Sequence abnormalities in the 5 known cofactors, 2 corepressors, and 3 coactivators were not identified (data not shown). However, a putative defect in one of the multiple proteins involved in regulation of nuclear receptor-mediated transcription (4) remains a distinct possibility to explain the observed greater severity of RTH β .

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Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1
Supplementary Table S1

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