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Research Article

Clinical Characteristics and Genotype-Phenotype Correlation in the Patients with the Diagnosis of Resistance to Thyroid Hormone Beta

Büyükyılmaz G et al. Clinical Characteristics of the *THRB* gene variants

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What is already known on this topic?
Variants in the *THRB* gene are the most common cause of resistance to thyroid hormone (RTH), defined as RTHβ. RTHβ is a rare condition, and mostly asymptomatic. Therefore, lack of awareness may lead to misdiagnosis, unnecessary tests or inappropriate management of the patient.

What this study adds?
In the present study evaluating the clinical and genetic characteristics of a series of 30 Turkish patients with genetically confirmed RTHβ in comparison to variant-negative patients, we expanded the *THRB* gene variant database with three additional novel variants. Besides, our results provide insights into prioritizing individuals for genetic analysis by comparing RTHβ patients with and without a variant.

Abstract
Objective: Resistance to thyroid hormone beta (RTHβ) is a rare disorder characterized by a fairly heterogeneous clinical presentation due to varying degrees of tissue response to thyroid hormone. The study aimed to evaluate the clinical, laboratory features and genotype-phenotype relationship of Turkish patients with RTHβ.
Methods: Patients who underwent a *THRB* gene analysis between September 2019 and September 2023 were retrospectively reviewed.
Results: 50 patients with the clinical features of RTHβ syndrome or a family history of an index case were included. A total of 8 different heterozygous pathogenic/likely pathogenic missense variants (3 novel) were detected in the *THRB* gene in 30 patients from 8 unrelated families. Although most patients with RTHβ were asymptomatic, 7 patients had various symptoms. Seven patients had received various treatments before diagnosis. Thyroid autoantibody was positive in 23% of all cases with a variant, and goitre was detected in 56% of children with a variant. While thyroid nodules were detected in 7 adult patients, two adult patients were being followed with papillary thyroid cancer. One child patient had attention-deficit disorder, learning disability, and type 1 diabetes mellitus. Of the 20 patients without a variant, TSHoma was detected in one.
Conclusion: The present study, provides an overview of clinical and genetic characteristics of patients with genetically confirmed RTHβ and expanded the *THRB* gene variant database with 3 novel variants. Although most patients with RTHβ are asymptomatic, molecular genetics analysis of the *THRB* gene and regular follow-up for potential concurrent autoimmune diseases and thyroid cancer is warranted.
Keywords: Thyroid hormones, Resistance to thyroid hormone, *THRB* gene, Autoimmune thyroid disease, Goitre

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Introduction

Defects in thyroid hormones (TH) (tetraiodothyronine (T4) and triiodothyronine (T3)) signaling, TH cell membrane transport, TH metabolism, or TH action lead to reduced TH sensitivity (1, 2). TH action defect is characterized by reduced response to circulating TH in target tissues defined as resistance to thyroid hormone (RTH). Variants in the thyroid hormone receptor gene account for the underlying etiology of RTH (3, 4). There are two distinct subtypes of the thyroid hormone receptor (TR); TRα and TRβ. Variants in the thyroid hormone receptor beta (*THRB*) gene are the most common cause of RTH, defined as RTHβ (5). The prevalence of RTHβ has been reported as 1 in 40,000 and 1 in 18,750 live births with no gender predominance (6, 7).
RTHβ is characterized by inappropriately normal or elevated thyroid-stimulating hormone (TSH) concentrations in the course of extremely elevated TH levels (3). RTHβ syndrome is mainly characterized by reduced effects of T3 at the cellular and tissue level (8). Excessive TH secretion usually compensates for the impaired sensitivity in peripheral tissues. Therefore, patients with RTHβ syndrome typically have an euthyroid state, thereby achieving normal growth and mental development. However, elevated TH may lead to thyrotoxic symptoms like tachycardia or irritability in some tissues, such as the heart, where TRα serves as a dominant receptor. Although rare, patients may experience clinical features of hypothyroidism, in cases with variants that severely abolish the TH receptor activity (1). These variable manifestations can be due to tissue variability in the RTH (9). The severity of the symptoms varies among the individuals, even those from the same family with an identical *THRB* gene variant (10).
TRβ is a ligand-dependent transcription factor consisting of two functional domains: the ligand-binding domain (LBD) at the carboxyl terminal, which recognizes T3, and the DNA-binding domain. The majority of the variants are located in three clusters enriched with CpG dinucleotide hot spots in the carboxy terminus of TRβ and result in mutant proteins (11). Although most cases have heterozygous variants, few cases have homozygous variants (4, 12). Most variants are single nucleotide substitutions leading to an amino acid change or, less frequently, to a truncated protein. Besides, nucleotide insertions, deletions, and duplications have also been described, resulting in frame-shift and nonsense variations (8). While 75% of cases with RTHβ syndrome have a dominant inheritance, it may also occur due to a *de novo* pathogenic variant (1). On the other hand, the underlying molecular defect can not be detected in about 15% of individuals with the RTHβ phenotype and this condition is referred to as "TR-RTH Unspecified" (13).
There are few studies evaluating the molecular genetics analysis of patients with RTHβ syndrome from Turkey. Firstly, Poyrazoglu et al. reported a variant in the *THRB* gene in a Turkish family (14). Following this report, Guran et al. reported the treatment outcome of their patient with the *THRB* gene variant (15). Isık et al. emphasized the underestimation of RTHβ diagnosis in a family whose index case was misdiagnosed as thyrotoxicosis and treated with antithyroid medication (16).
As RTHβ is rare, and most patients are asymptomatic lack of awareness may lead to misdiagnosis, unnecessary tests, or inappropriate patient management. The present study aimed to evaluate the clinical, laboratory features, and genotype-phenotype relationship of a series of Turkish patients with RTHβ syndrome due to *THRB* gene variants.

Patients and Methods

Patients

A retrospective examination was conducted on all patients and their families who underwent *THRB* (NM_001354712.2) gene analysis at Ankara Bilkent City Hospital Pediatric Endocrinology Clinic, and Ankara Bilkent City Hospital Endocrinology and Metabolism Clinic with a presumptive diagnosis of RTHβ between September 2019 and September 2023. The study was approved by the Clinical Research Ethics Committee of Ankara Bilkent City Hospital with

decision number 23-5676/22.11.2023. The chief complaints, age of the diagnosis, sex, past treatments, body weight, height, body mass index (BMI), standard deviation scores (SDS), pulse rate, serum TSH, free T4 (FT4), and free T3 (FT3) concentrations, anti-thyroglobulin antibody (Tg-Ab), and antithyroid peroxidase antibody (TPO-Ab) results of the patients were extracted from the patients' files. Pituitary magnetic resonance imaging (MRI) findings, thyroid ultrasound, echocardiography, and genetic analysis results were evaluated. We double-checked the thyroid function tests of all patients undergoing molecular genetic analysis, as lab and assay-specific variations could potentially affect correct phenotyping and thus pick-up rate for a variant. Besides, none of the patients from our cohort were using biotin or any medication that could interfere with the thyroid hormone measurement.

All patients were evaluated for goitre. In children, the volume of each lobe was calculated using the formula of length × width × depth × 0.52. The thyroid volume was determined as the sum of both lobes and then SDS were calculated using age- and sex-specific references (17). Values above 2 SDS were considered goitre in children. In adults, the thyroid volume of each lobe was calculated using the formula of V (ml)=0.479× width (cm)×depth (cm)×length (cm) (18). A thyroid volume above 10 ml in women and 15 ml in men was considered goitre (19).

The hormonal profile of patients with a pathogenic or likely pathogenic variant was compared with age and sex-matched healthy control. The FT4/FT3 ratio was calculated after unifying FT4 and FT3 units as pmol/L.

Molecular Genetic Analyses:

Genomic DNA was extracted from peripheral blood leukocytes. For the index patients, all of the coding exons and exon-intron boundaries of the *THRB* gene were amplified by specific primers via polymerase chain reaction (PCR). After cycle sequencing all PCR products were purified and sequenced on ABI 3100 Genetic Analyzer (Applied Biosystems®). All the sequences aligned to the reference genome and analysed in SeqScape ® Software (ABI, California, USA). For the relatives of the probands, we used only variant-associated exonic primers and analysed them with Sanger sequencing. All variants were interpreted according to the American College of Medical Genetics and Genomics Guidelines (20). Written informed consent was obtained from all patients/their legal guardians.

Statistical analysis

Statistical analysis was evaluated using the IBM-SPSS 24.0 (IBM Corporation, Armonk, NY, USA) program. The mean and standard deviation (SD), median and quartile values of numerical variables were calculated. Categorical variables were expressed as frequency and percentages (%). Shapiro Wilk test was used to evaluate the normal distribution of data. Normally distributed numerical variables were evaluated using the Student's T-test, and the Mann-Whitney U test was used if the parametric test assumptions were not met. Chi-square analysis and Fisher Exact tests were used to compare categorical variables. A p-value less than 0.05 was considered as statistical significance.

Results

The study included 50 patients admitted with the clinical features of RTHβ syndrome or a family history of an index case. All participants underwent *THRB* gene analysis. Thirty patients from 8 unrelated families were found to have pathogenic/likely pathogenic variants in the *THRB* gene. Clinical characteristics and hormonal features of patients with a pathogenic variant are shown in Table 1. Variant characteristics and classifications detected in our cohort are displayed in Table 2.

The number of children and adults with a pathogenic or likely pathogenic variant was 16 (F/M: 11/5) and 14 (F/M:7/7), respectively. While 12 of these adults were family members of our pediatric patients, families (family 7 and 8) of two adult patients could not be evaluated because they were either deceased or unwilling to participate in the study. Besides, the thyroid function test values and molecular genetic analysis results of the parents of children with a likely pathogenic variant in Family 6 were normal suggesting a *"de novo"* variant. The pedigrees of families 1, 2, 3, 4, 5, and 6 are presented in Figure 1. The median (1st and 3rd quartile) age in children with a pathogenic or likely pathogenic variant was 9.7 (5.2-11.4) years, and the median (1st and 3rd quartile) age in adults with a pathogenic or likely pathogenic variant was 36 (33.8-46) years. All children had a height SDS above −2SD. BMI-SDS was within the normal range in all children except for two cases with malnutrition (case III-6 from family 2 and III-1 from family 5) (Table 1). One of the children (III-1 from family 5) had a diagnosis of type 1 diabetes mellitus (T1DM). While 23 of the 30 patients were asymptomatic, 7 had various symptoms (two patients had palpitations, two had goitre, one had sweating, one had anxiety, and one had attention-deficit disorder (ADD), and learning disability (LD)). Seven (25%) patients had received various forms of treatment at other centres before the diagnosis of RTHβ (Table 1).

Twenty-six patients with a pathogenic or likely pathogenic variant could be evaluated for autoimmune thyroiditis. Six (from 4 different families) (23%) of 26 patients had autoimmune thyroiditis. Of those all were females. There was no statistically significant difference between TSH (p = 0.466), FT4 (p = 0.420), and FT3 (p = 0.168) levels of patients with negative or positive thyroid autoantibody.

In the RTHβ group, thyroid volume was above 2 SDS in 9 (56%) children. Thyroid nodules were found in 7 adult patients. In one patient (II-2 from family 4), a fine-needle aspiration biopsy (FNAB) revealed benign cytology. In family 2, three individuals (I-2, II-1, and II-5) underwent total thyroidectomy. Of those two, (I-2 and II-1) had papillary thyroid carcinoma (PTC) while patient II-5 had a benign cytology (21). A pituitary MRI, performed on four adult patients with a pathogenic or likely pathogenic variant, revealed pituitary incidentaloma in one and normal MRI in three patients.

A total of 8 different heterozygous variants (3 novel) were detected in 8 families. All variants were missense resulting from a single nucleotide change. Three variants were in Cluster 3, four in Cluster 2, and only one in Cluster 1.

No variant was detected in the *THRB* gene in 20 patients. While 17 of the 20 patients were examined because of clinical and/or laboratory findings suggesting RTHβ, the remaining three euthyroid individuals were examined as part of family screening. Clinical characteristics and hormonal features of 17 patients (8 children, 9 adults) who underwent genetic analysis but no variant was detected are shown in Supplementary Table 1. Of the 17 patients without a variant, 16 were assessed for autoimmune thyroiditis. Out of these 16 patients, only one (7%) adult female had autoimmunity. The thyroid volume could be calculated in all children but seven adult patients with no variant. In the pediatric group, thyroid volume was above 2 SDS in 3 children (37%), whereas, among adults, 4 individuals (57%) had goitre. Out of nine adult patients, seven underwent an MRI scan. In one patient, a TSH-producing pituitary adenoma (TSHoma) was detected, while in another patient, a nonfunctional adenoma (incidentaloma) was detected. Since there was no Multiplex Ligation-dependent Probe Amplification (MLPA) kit available for *THRB*, two patients without detected variants underwent high-resolution array comparative genomic hybridization (CytoScan HTCMA_96r3.1, Thermo Fisher Scientific, Waltham, MA). No pathology was detected.

Comparison of the anthropometric and laboratory findings of patients with a variant (n: 30) and without a variant but having clinical/laboratory features of RTHβ (n:17) are summarized in Table 3. In the pediatric group, patients with a variant had statistically significantly higher FT4 (p = 0.004) and FT3 (p = 0.009) than the variant-negative group, while no difference was observed in TSH levels (p = 0.287). Remarkably, in the adult group, while there was no statistically significant difference between FT3 (p = 0.099) and FT4 (p = 0.088) levels of patients with and without variants, TSH levels were higher in patients with variants; compared to the patients without variants ((4.06±2.28 mIU/mL vs. 1.95±0.80 mIU/mL), p = 0.016). Besides, there was no statistically significant difference between the FT4/TSH, FT4 (pmol/L) / FT3 (pmol/L), and FT3 (pmol/L) / FT4 (pmol/L) ratios of patients with and without a variant (Table 3).

Comparison of the laboratory findings of patients with a variant and healthy control group are summarized in Table 4. There was no statistically significant difference between TSH values in children. However, TSH levels were higher in adults with a variant. In addition, while the FT4/TSH ratio was higher in children with a variant, no difference was detected between the FT4 (pmol/L) / FT3 (pmol/L) (children, p = 0.868; adult, p = 0.053) and FT3 (pmol/L) / FT4 (pmol/L) ratios (children, p = 0.877; adult, p = 0.054) between the patients with a variant and healthy controls.

A comprehensive cardiological evaluation was performed for all pediatric patients regardless of variant status. We did not detect any abnormality in either echocardiography or electrocardiography of the patients including the holter monitorization performed in 9 children.

Discussion

In the present study, evaluating *THRB* gene analysis in a series of 50 patients with signs and symptoms of RTHβ syndrome or a history of the index case in their families, we detected 8 variants (3 novel) in 30 out of 50 individuals.

Clinical manifestations of RTHβ syndrome exhibit a wide range. Although euthyroid status can be achieved in most patients with high TH values appropriate to the level of mutant receptors in the tissues, phenotypes of the patients vary depending on the location of the hormonal resistance (9). The most common symptoms reported in the literature are goitre (65%–85%), tachycardia (33%–75%), ADD, and LD (33%–68%), respectively (8, 22, 23). Lessly reported symptoms were increased incidence of speech disorder, short stature, increased frequency of ear, nose, and throat infections, underweight in children, hearing loss, and cardiac abnormalities (23). Consistently, in our series, most of our patients with a pathogenic or likely pathogenic variant were asymptomatic. RTHβ syndrome due to single amino acid changes in the THRB gene is reported to be milder than those due to insertion, deletion, or truncation variants (8, 24). In our series, all patients with a pathogenic or likely pathogenic variant had missense heterozygous variants. We, therefore, attributed the high rate of asymptomatic cases in our series to the presence of missense variants as underlying molecular genetics aetiology.

In a study evaluating RTHβ patients, 41.7% of the patients were shown to receive inappropriate treatments, including antithyroid therapy, thyroidectomy and radio-iodine ablation (25). While treatment is recommended for symptomatic cases, except for limited experiences with TH analogues (triiodothyroacetic acid, TRIAC), there is no specific treatment option for RTHβ syndrome patients (26, 27). Nevertheless, the rate of inappropriate treatment tends to decrease compared to previous publications, as reported in our study can be attributed to the increased awareness of RTHβ and the opportunity to access molecular genetics analysis.

The increased prevalence of goitre despite mostly normal TSH has been reported to be due to alterations in terminal sialic acid residues, which enhance the biological potency of TSH (28). In our series, diffuse goitre was more prevalent in pediatric patients with a variant (56%) than in patients without a variant (37%).

TSHoma was detected in one of 17 patients with clinical/laboratory findings of RTHβ whilst no variants were detected in the *THRB* gene. There was no identified cause in the remaining 16 patients**.** The inability to explore the underlying aetiology in these patients might be due to several factors such as lack of facility to conduct further investigations and genetic analyses, errors in laboratory tests, the possible presence of somatic mosaicism, the existence of variants not covered by coding region sequencing (deep intronic variants, variants in inter or intragenic regions that regulate gene expression, etc), or may be due to new modifier genes that has not yet been identified (1, 4). Indeed, mosaicism in RTHβ was first reported by Mamanasiri et al. who did not detect a variant in the *THRB* gene in 15% of individuals who had the RTHβ phenotype (29). Lack of measurement of serum biomarkers of TH effects on peripheral tissues such as cholesterol, creatine kinase, alkaline phosphatase, osteocalcin and sex hormone binding globulin might be the limitation of our study. However, Refetoff et al. reported that these values are less reliable unless measured before and after administration of T3 (3).

The number of studies comparing patients with and without RTHβ is scarce. In the study of Brucker-Davis et al., individuals with RTHβ were younger, exhibited a higher rate of palpable goitre, had shorter stature, lower body weight, lower IQ scores, higher FT3 and FT4 levels, and higher T4/TSH and T4/T3 ratios (23). In our series, in children with a variant, FT3 and FT4 values were higher than those without variants, while in the adult group, no differences were observed in these values**.** Besides, FT4/TSH, FT4/FT3, and FT3/FT4 ratios of patients with and without a variant did not differ. Compared to the healthy controls TSH levels were not different in children but it was higher in adults with a variant. Also, the FT4/TSH ratio was higher in children with a variant whilst no difference was detected between the FT4/FT3 and FT3/FT4 ratios. This finding was consistent with the results of Refetoff et al. indicating the total T3/total T4 ratio of patients with generalized RTHβ was only slightly above the mean value found in euthyroid-healthy individuals (3).

Individuals with RTHβ have been reported to have a higher likelihood of developing AITD (30). In the present study, the rate of AITD was 23% in RTHβ and all cases were female which was consistent with the previously reported female predominance (25). While Gavin et al. suggest that high TSH in RTHβ might activate intrathyroidal lymphocytes and increase proinflammatory cytokines and thyroid cell destruction, Barkoff et al. reported that this hypothesis does not explain the increased autoimmunity in RTHβ (31). Moreover, the role of TH on the immune system is still poorly understood, and TH is reported to activate the immune system by acting directly on thymic epithelial cells, neutrophils, natural killer cells, macrophages, and dendritic cells (9, 32, 33). Besides, while there is a female predominance in thyroid autoimmunity, there is no sex difference in RTHβ. However, all patients with thyroid autoimmunity were female in our series and some of the other studies suggest a need for further investigation of the mechanism behind the association between AITD and RTHβ which remains unclear. RTHβ has been reported in patients with renal failure, ichthyosis-eczema, psychotic attacks, oesophagal atresia, reflux, celiac disease, congenital heart disease, T1DM and T2DM (23, 26, 34, 35). One patient in our series was also diagnosed with T1DM. TRs such as TRα1 and TRβ1 have been shown expressed in pancreatic beta cells (36). Additionally, it has been reported that T3 induces the proliferation of pancreatic β-cells by activating phosphoinositide 3-kinase/Akt and kinase pathways. Therefore, T3 could be considered a survival factor for islet cells, by protecting them from apoptosis (37). Except for our case, T1DM has been reported in two other cases. There is no evidence to consider whether this association is coincidental or not. Although the results of studies evaluating the effects of TH on insulin secretion are controversial, the effect of mutant TH receptors on islet cell function is not fully understood, and assessment of glucose metabolism in these patients is warranted (38, 39).

Studies investigating the role of TH receptors in cancer have argued that decreased TR gene expression in cancer tissues due to hypermethylation or TR gene deletions can be explained by the potential tumour-suppressive function of TRs. Furthermore, these studies have highlighted the association of somatic variants in TRs with human cancers suggesting that the loss of normal TR function might lead to uncontrolled cell growth and poor differentiation (40). In 2001, Taniyama et al. reported the first case of RTHβ associated with PTC. In 2022, Fang et al. published a literature review of 17 cases including their case (41, 42). Two patients in family 2 investigated in the present study were also reported in this series (21).

Our study has some limitations. First, the sample size was relatively small. Due to the low frequency of RTHβ, further multicenter or nationwide studies with larger sample sizes are needed to elucidate the clinical characteristics and genotype–phenotype association of RTHβ. Second, in some patients, in whom we could not detect the *THRB* gene variant, further investigations using advanced genetic and laboratory analysis methods could not be performed.

In conclusion, in the present study evaluating the clinical and genetic characteristics of a series of 30 Turkish patients with genetically confirmed RTHβ, we expanded the *THRB* gene variant database with 3 novel variants. Besides, our results provide insights into prioritizing individuals for genetic analysis by comparing RTHβ patients with and without a variant. Although most patients with RTHβ are asymptomatic, prompt molecular genetic analysis for *THRB* gene variants and regular follow-up for potential concurrent autoimmune diseases and thyroid cancer is warranted.

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Compliance with Ethical Statements

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All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Author Contribution

- Gonul Buyukyilmaz, Keziban Toksoy Adiguzel, Oya Topaloglu, Busra Erozan Cavdarlı, Cevdet Aydın, Sema Hepsen, Erman Cakal, Nur Semerci Gunduz, Fatih Gurbuz, Mehmet Boyraz, Serkan Bilge Koca, Hüseyin Demirbilek were responsible for medical practices, data collection and processing.
- Gonul Buyukyilmaz, Busra Erozan Cavdarlı, and Huseyin Demirbilek were responsible for the conceptualization of the study.
- Gonul Buyukyilmaz, Serkan Bilge Koca, Busra Erozan Cavdarlı, Nur Semerci Gunduz, Erman Cakal and Huseyin Demirbilek were responsible for the analysis and interpretation of the data.
- Gonul Buyukyilmaz, Serkan Bilge Koca, Oya Topaloglu, Cevdet Aydın, Nur Semerci Gunduz and Huseyin Demirbilek were responsible for the literature search.
- Gonul Buyukyilmaz, Serkan Bilge Koca, Busra Erozan Cavdarlı, and Huseyin Demirbilek were responsible for writing the draft manuscript.
- All authors participated in the final read and approved the final version of the manuscript. Huseyin Demirbilek critically revised the final draft of the manuscript before submission.

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Figure 1 Schematic presentation of the chromosomal location, exon-intron organization, and protein domain content of the *THRB* gene. The detected variants have been aligned on the exonic and cluster levels. On the pedigrees of familial cases; black-filled squares and circles indicate affected individuals, and those marked with an asterisk indicate individuals with *THRB* gene analysis

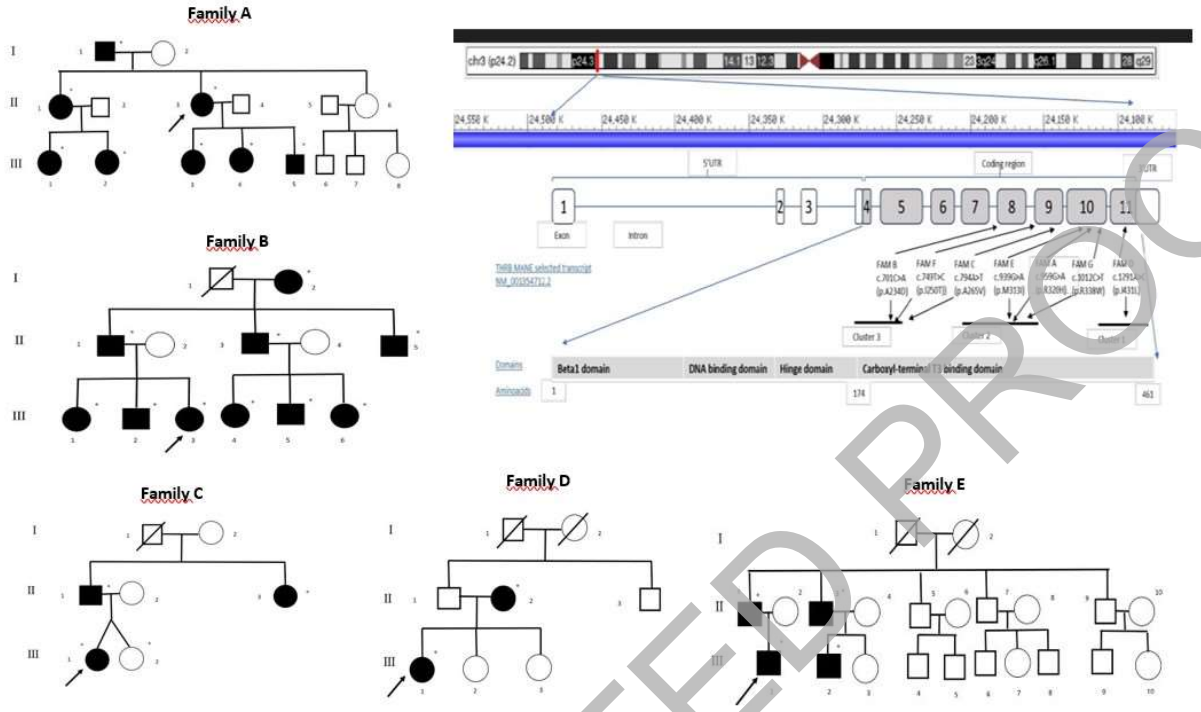

Figure 1: Schematic presentation of the chromosomal location, exon-intron organization, and protein domain content of the THRB gene. The detected variants have been aligned on the exonic and cluster levels. On the pedigrees of familial cases; black-filled squares and circles indicate affected individuals, and those marked with an asterisk indicate individuals with THRB gene analysis.

Table 1: Clinical and laboratory findings of the patients with THRB gene pathogenic or likely pathogenic variants.

Family No	Individual	Sex	Age at dx years	Initial presentation	Previous treatment	Tachycardia	Height cm (SDS) BMI (SDS)	FT3 ng/L (Adult: 2.3-4.2) (children: 3-4.7)	FT4 ng/dL (Adult: 0.89-1.76) (children: 0.83-1.43)	TSH mU/L (0.5 - 4.78)	Autoimmunity markers	Thyroid volume (SDS or mL)	Thyroid nodule	Pituitary MRI	Other clinical features
1	I-1	M	55	Asymptomatic	None	No	NA	NA	NA	NA	NA	NA	NA	No	NA
	II-1	F	36	Asymptomatic	None	No	153 25.6	4	1.74	0.72	NA	NA	NA	No	(-)
	II-3	F	35	Palpitation	Triiodothyronine sodium	Yes	157 28.3	4.7	1.6	5.4	Anti-tpo + Anti tg-	13.1 ml	Multinodular	No	(-)
	III-1	F	10.5	Asymptomatic	None	No	142.7 (0.14) 15.9 (-0.71)	7.5	1.98	1.4	Anti-tpo + Anti-tg+	2.3 SDS	No	No	(-)
	III-2	F	6.9	Asymptomatic	None	No	115 (-1) 13.6 (-1.3)	8.3	2.7	1.3	Negative	2.2 SDS	No	No	(-)
	III-3	F	9.9	Asymptomatic	None	No	127.7 (-1.5) 13.9 (-1.62)	6	1.9	1.8	Negative	-0.15 SDS	No	No	(-)
	III-4	F	4.9	Asymptomatic	None	No	104.5 (-0.66) 14.8 (-0.43)	7.3	1.9	4.1	Negative	1.1 SDS	No	No	(-)
	III-5	M	13.5	Asymptomatic	None	No	153 (-0.97) 15.4 (-1.86)	7.3	1.7	1.6	Negative	2.1 SDS	No	No	(-)
2	I-2	F	56	Goitre	NA	No	153 38.4	6.2	2.45	2.81	Negative	NA	Total thyroidectomypapillar thyroid cancer	No	Type 2 DM
	II-1	M	33	Asympotomatic	None	No	178 24.9	7.2	1.95	5.52	Negative	NA	Total thyroidectomypapillar thyroid cancer	No	(-)
	II-3	M	40	Asympotomatic	None	No	NA	NA	NA	NA	NA	NA	NA	No	NA
	II-5	M	33	Asympotomatic	None	No	175 24.4	6.9	1.98	3.1	Negative	NA	Total thyroidectomybenign multinodular goitre	No	(-)
	III-1	F	11.5	Asymptomatic	Propranolol, mmz	No	152 (0.36) 21.9 (1)	9	2.2	3.8	Negative	9.8 SDS	No	No	(-)
	III-2	M	17.1	Asymptomatic	None	No	178 (0.57) 27.1 (1.38)	8.9	2.1	3.3	negative	2.64 SDS	No	No	(-)
	III-3	F	10.1	Sweating	Propranolol	No	144.7 (0.95) 17.1 (0.01)	7.6	2.5	1	Anti-tpo+ Anti-tg+	9 SDS	No	No	(-)
	III-4	F	1.5	Asymptomatic	None	No	80 (0.49) 16.1 (-0.08)	9.4	2.2	2.5	Negative	1.1 SDS	No	No	(-)
	III-5	M	1.1	Asymptomatic	None	No	75.8 (-0.67) 16.3 (-0.51)	10.9	2.6	3.9	Negative	2 SDS	No	No	(-)
	III-6	F	7.8	Asymptomatic	None	No	119 (0.89) 16.4 (-2.46)	7.8	2.4	3.3	Negative	2.5 SDS	No	No	(-)
3	II-1	M	36	Goitre	None	No	174 27	5.77	2.68	3.2	Negative	NA	Multinodular, FNAB recommended	No	(-)

	II-3	F	19.5	Asymptomatic	L-T4	No	157 20.6	6.8	2.7	5.8	Negative	NA	No	Yes, normal	(-)
	III-1	F	1.1	Asymptomatic	None	No	73 (-1.09) 19.1 (1.58)	8.57	2.54	3.6	Negative	1 SDS	No	No	Premature
4	II-2	F	40	Asymptomatic	None	No	155 36.6	7.9	2.7	7.9	Anti-tpo+ Anti-tg+	18.7 mL	Multinodular FNAB:Benign	Yes, microadenom	(-)
	III-1	F	16.9	Asymptomatic	None	No	152 (-1.82) 25.3 (1.4)	5.5	2.6	2.4	Anti-tpo+ Anti-tg+	5,8 SDS	No	No	(-)
5	II-1	M	36	Asymptomatic	None	No	170 24.2	7.8	2.8	2.6	NA	NA	NA	No	(-)
	II-3	M	43	Asymptomatic	Proprano lol	No	170 20.7	5.51	2.57	1.75	Negative	NA	No	Yes, normal	(-)
	III-1	M	6	ADD, LD	Proprano lol	No	108.5 (-1.45) 12.4 (-2.64)	6.6	2.7	2	Negative	1,8 SDS	No	No	Type 1 DM
	III-2	M	11	Asymptomatic	None	No	143 (-0.15) 25.4 (1.8)	9.47	2.79	3.9	Negative	1.68 SDS	No	No	(-)
6	II-1	F	9.5	Anxiety disorder	None	No	137.7 (0.47) 20.4 (1.27)	7.43	2.13	2.96	Negative	1.17 SDS	No	No	(-)
7		F	20.5	Palpitation	Proprano lol	Yes	160 23.4	8.6	4	3	Negative	NA	No	No	NA
8		F	53	Asymptomatic	NA	No	NA	5.62	2.26	7.9	Anti tpo+ Anti tg-	NA	Multinodular	Yes, normal	NA

Age at dx: Age at diagnosis, SDS: Standard deviation score, NA: Not available, SDS: standard deviation score, BMI: Body mass index, FNAB: Fine-needle aspiration biopsy. FT3: Free triiodothyronine, FT4: Free tetraiodothyronine, TSH: Thyroid-stimulating hormone, Mmz: Methimazole, LT4: Levothyroxine sodium, Anti –TPO: Antithyroid peroxidase, Anti-tg: Anti-thyroglobulin, DM: Diabetes mellitus, MRI: Magnetic resonance imaging, ADD: Attention-deficit disorder, LD: Learning disability

Table 2: *THRB* gene variants detected in the families and variant classification according to the guidelines

Family	Mutation (cDNA/ protein)	Cluster Region/domain	Status	ACMG classification	Inheritance
1	c.959G>A (p.R320H)	2	Clinvar-RCV000760097	Pathogenic; PS3, PM5, PM1, PM2	Familial
2	c.701C>A (p.A234D)	3	Reported by literature[43]	Likely pathogenic; PM5, PM1, PM2, PP3	Familial
3	c.794A>T (p.D265V)	3	Novel	Likely pathogenic; PM1, PM2, PP3-S	Familial
4	c.1291A>C (p.I431L)	1	Novel	Likely pathogenic; PM5, PM1, PM2, PP3	Familial
5	c.939G>A (p.M313I)	2	Novel	Likely pathogenic; PM5, PM1, PM2, PP3	Familial
6	c.749T>C (p.I250T)	3	Clinvar- RCV000760094	Likely pathogenic; PM1, PM2, PP3, PP5-M	De novo
7	c.1012C>T (p.R338W)	2	Clinvar- RCV000013385	Pathogenic; PS3, PM1, PM2, PP3-S	Unknown
8	c.980C>A (p.T327N)	2	Clinvar-RCV000582153	Likely pathogenic, PM1, PM2, PP3-S, PP5	Unknown

Table 3: Comparison of the anthropometric and laboratory findings of the patients with a variant and without a variant but having clinical/laboratory findings similar to RTHβ.

	Children *THRB+*	Children *THRB-*	P value	Adult *THRB +*	Adult *THRB-*	P value	All group mutation+	All group mutation-	P value
Female/Male	11/5	3/4	0.363$^{\phi}$	7/7	2/8	0.210$^{\phi}$	18/12	5/12	0.044*
Age (year)	9.7 (5.2-11.4)	9.5 (8.3-11.9)	0.664$^{\Psi}$	36 (33.8-46)	27 (22.6-45.5)	0.253$^{\Psi}$	17 (9.1-36)	18.4 (10.4-33.5)	0.690$^{\Psi}$
Height SDS	-0.34±0.91	0.32±1.28	0.245$^{\mu}$	164.50±9.85	NA	NA	NA	NA	NA
BMI SDS	-0.19±1.49	0.11±1.00	0.564$^{\mu}$	26.96±6.15	NA	NA	NA	NA	NA
FT3	7.99±1.38	6.17±1.25	**0.009**$^{\mu}$	6.40±1.39	5.40±1.22	0.099$^{\mu}$	7.31±1.58	5.74±1.25	**0.001**$^{\mu}$
FT4	2.32±0.34	1.87±0.15	**0.004**$^{\mu}$	2.46±0.64	2.07±0.26	0.088$^{\Psi}$	2.38±0.48	1.98±0.25	**0.002**$^{\Psi}$
TSH	2.68±1.04	2.23±0.50	0.287$^{\mu}$	4.06±2.28	1.95±0.80	**0.016**$^{\mu}$	3.27±1.79	2.07±0.68	**0.014**$^{\Psi}$
Autoimmunity	3	0	NA	3 (33.3%)	1 (12.5%)	0.576$^{\phi}$	6 (24%)	1 (6.7%)	0.224$^{\phi}$
Thyroid SDS	2.87±2.83	0.15±0.58	**0.02**$^{\Psi}$	NA	NA	NA	NA	NA	NA
FT4/TSH	1.03±0.54	0.87±0.20	0.894$^{\Psi}$	0.87±0.63	1.26±0.68	0.136$^{\Psi}$	0.96±0.57	1.09±0.55	0.242$^{\Psi}$

FT4/FT3	2.96±0.67	3.16±0.91	0.662[Ψ]	3.88±0.67	4.20±1.21	0.859[Ψ]	3.35±0.80	3.74±1.18	0.479[Ψ]
FT3/FT4	**0.35±0.06**	**0.33±0.07**	**0.635[µ]**	**0.27±0.05**	**0.26±0.05**	**0.905[µ]**	**0.31±0.07**	**0.29±0.07**	**0.386[µ]**

[*] Chi Square test, [ϕ] Fisher's Exact Test, [µ] Student's T test, [Ψ] Mann Whitney U test.

Data are presented as mean±SD, or median (Q1-Q3), BMI: Body mass index, SDS: standard deviation score, NA: not available, FT3: Free triiodothyronine, FT4: Free tetraiodothyronine, TSH: Thyroid-stimulating hormone, RTHβ: Resistance to thyroid hormone β

Tablo 4. Comparison of the anthropometric and laboratory findings of the patients with a detected variant of *RTHB* and healthy controls.

	Children *THRB*+	Children control	*P*-value	Adult *THRB*+	Adult control	*P*-value
Female/Male	11/5	14/10	0.505[*]	7/7	9/14	0.517
Age (year)	9.7 (5.2-1.4)	9.4 (5.6-11.1)	0.945[Ψ]	36 (33.8-46)	35 (25-43)	0.316[Ψ]
FT3	8±1.38	3.94±0.22	**<0.001[µ]**	6.41±1.40	3.51±0.38	**<0.001[µ]**
FT4	2.32±0.35	1.13±1.12	**<0.001[µ]**	2.46±0.64	1.18±0.15	**<0.001[µ]**
TSH	2.69±1.05	2.88±1.2	0.605[µ]	4.07±2.29	1.89±1.00	**0.002[Ψ]**
FT4/TSH	1.03±0.54	0.45±0.16	**<0.001[Ψ]**	0.88±0.63	0.78±0.35	0.972[Ψ]
FT4/FT3	2.96±0.67	2.89±0.34	0.868[Ψ]	3.88±0.67	4.36±0.63	0.053[µ]
FT3/FT4	0.35±0.06	0.35±0.04	0.877[µ]	0.27±0.05	0.30±0.04	0.054[µ]

[*] Chi Square test, [µ] Student's T test, [Ψ] Mann Whitney U test.

Data are presented as Mean ± SD and (median (Q1-Q3). FT3: Free triiodothyronine, FT4: Free tetraiodothyronine, TSH: Thyroid-stimulating hormone, RTHβ: Resistance to thyroid hormone β

Supplementary Table 1. Clinical and laboratory findings of the patients with a clinical prediagnosis of RTHβ and without *THRB* gene variant

Patient No	Sex	Age years	Initial presentation	Previous treatment	Tachycardia	Height (cm (SDS)) BMI **(SDS)**	FT3 ng/L (Adult: 2.3-4.2) (children: 3-4.7)	FT4 ng/dL (Adult: 0.89-1.76) (children: 0.83-1.43)	TSH mU/L (0.55-4.78)	Autoimmunity	Thyroid volume (SDS or mL)	Thyroid nodule	Pituitary MRI	Other clinical features
1	M	9.5	Asymptomatic	None	No	151 (2.8) 18.2 (0.59)	8.29	1.95	2.9	Negative	3.17 SDS	No	No	CNV normal
2	M	13.4	Asymptomatic	None	No	157.7 (-1.45) 18 (-1.1)	6.59	1.72	1.9	Negative	-0.97 SDS	No	No	(-)
3	F	39	Palpitation	Proprapranolol	Yes	NA	4.5	1.88	3.57	Negative	12.8 ml	Isohypoechoic nodule at follow-up	Normal	CNV normal
4	M	28	Palpitation	Bisoprolol fumarate	Yes	NA	5.92	2.17	1.96	Negative	25.9 ml	No	Tshoma	(-)
5	M	18.4	Palpitation	Mmz	Yes	NA	4.79	1.67	1.34	Negative	11.4 ml	No	Normal	(-)
6	F	25	Asymptomatic	None	No	NA	3.09	2.21	2.07	Anti-tpo+ Anti-tg+	6.3 ml	No	Normal	(-)
7			...matic			129.5 (0.14) 20.2 (1.5)	6.5	2.1	2.6	Negative	-0.27 SDS	No	No	(-)
8	M	44	Asymptomatic	None	No	NA	6.4	2.1	1.5	NA	NA	NA	No	(-)
9	M	11.9	Asymptomatic	None	No	150.7 (0.11) 15.9 (-1.24)	4.09	2.08	1.9	Negative	0.01 SDS	No	No	(-)
10	M	18	Asympotomatic	None	No	n/a	5.08	2.1	0.78	Negative	2.2 SDS	No	No	(-)

11	M	24	Asympoto matic	None	No	171 19.8	5.3	2.5	1.6	Negative	7.4 ml	No	Normal	(-)
12	F	3.2	Palpitation	Proprano lol	Yes	114.8 (0.24) 15.7 (0.13)	6.1	1.7	2.7	Negative	-0.13 SDS	No	No	(-)
13	M	26	Asympto matic	None	No	NA	6.1	2.3	1.6	Negative	19.2 ml	No	Normal	(-)
14	M	8.3	Asympto matic	None	No	132 (0.71) 18 (0.9)	5.9	1.9	1.6	Negative	1.27 SDS	No	No	(-)
15	F	11.3	Asympto matic	None	No	145.5 (-0.26) 18.3 (0.03)	5.7	1.6	1.8	Negative	0.6 SDS	No	No	(-)
16	M	50	Asympto matic	None	No	NA	7.2	2.4	2.2	Negative	19.8 ml	Isoechoic nodule FNAB: Benign	Microad enoma	(-)
17	M	3	Asympto matic	None	No	90 (-0,86) 17.9 (1.1)	7.2	1.9	1.9	Negative	3.7 SDS	No	No	(-)

SDS: Standard deviation score, NA: Not available, BMI: Body mass index, FNAB: Fine-needle aspiration biopsy. FT3: Free triiodothyronine, FT4: Free tetraiodothyronine, TSH: Thyroid-stimulating hormone, Mmz: Methimazole, Anti –TPO: Antithyroid peroxidase, Anti-tg: Anti-thyroglobulin, , MRI: Magnetic resonance imaging, CNV: Copy number variant, RTHβ: Resistance to thyroid hormone β