Autoimmune thyroid disease and thyroid function test fluctuations in patients with resistance to thyroid hormone

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Abstract

Objective: Resistance to thyroid hormone beta (RTH β) is an inherited syndrome caused by mutations in the thyroid hormone receptor β (*THRB*) gene. Patients with RTH β typically have elevated thyroid hormone levels with non-suppressed serum thyroid-stimulating hormone (TSH). We aimed to elucidate the clinical, laboratory, and imaging findings of RTH β patients and further to explore their association with *THRB* gene mutations.

Design and methods: We retrospectively reviewed the clinical charts and compared the clinical findings of 68 RTH β patients (45 probands and 23 relatives) and 30 unaffected relatives in Kuma Hospital.

Results: Genetic testing revealed 35 heterozygous *THRB* gene mutations. Among all RTH β patients, autoimmune thyroid disease (AITD) was detected in 42.1% of men and 40.9% of women, showing that the prevalence of AITD in affected males was significantly higher than in unaffected relatives ($P = 0.019$). During the follow-up of 44 patients, 13 patients (29.5%; 8 (42.1%) with AITD and 5 (20%) without AITD) temporarily showed thyroid function test results inconsistent with RTH β . Two patients with the R383H mutation, which has little dominant-negative effect, temporarily showed normal thyroid hormone and TSH levels without AITD.

Conclusions: The frequency of AITD in male RTH β patients was significantly higher compared to unaffected relatives. More than 20% of RTH β patients temporarily showed laboratory findings atypical of RTH β during their follow-up, and patients with AITD and specific *THRB* mutations were prone to display such findings. Therefore, genetic testing should be performed even for patients with fluctuations in thyroid function test results to avoid misdiagnosis and inappropriate treatment.

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Introduction

Resistance to thyroid hormone beta (RTH β) is an inherited condition, which was first described by Refetoff *et al.* (1). Mutations in the thyroid hormone receptor β (*THRB*) gene have been identified in the majority of patients with RTH β , with more than 4000 patients belonging to over 600 families (2). In general, patients with RTH β are identified using discrepant thyroid function test and elevated serum levels of free T4 (FT4) and free T3 (FT3) with non-suppressed thyroid-stimulating hormone (TSH). Most studies

involving a large number of patients with RTH β originate from North America and Europe (3, 4, 5). However, no studies involving a large series of patients with RTH β have been conducted in Asian countries including Japan.

Barkoff *et al.* reported that 23.3% of patients with RTH β had a comorbid autoimmune thyroid disease (AITD). They described that individuals with RTH β have an increased likelihood of developing AITD (6). Vela *et al.* (4) and Rivolta *et al.* (7) reported that thyroid autoantibodies were

found in 25 and 22.2% of patients with RTH β in Spain and South America, respectively. However, these studies did not investigate the increase in thyroid autoantibodies in patients with RTH β in comparison to a control. A study of the general Japanese population reported that thyroid autoantibodies were detected in 23.4% of women and 14.8% of men with non-palpable goiters (8). No study has reported the prevalence of AITD among Japanese patients with RTH β .

The main purpose of this study was to evaluate the clinical, laboratory, and imaging findings of patients with RTH β in Japan, an iodine-sufficient or even excess area, and further to explore their association with *THRB* gene mutations.

them, 45 patients were confirmed to have *THRB* mutations. Relatives of these patients underwent gene analysis to determine if any of them also had *THRB* mutations. *THRB* gene sequencing confirmed mutations in 23 and normal sequences (WT) in 30 relatives. Overall, the study included 98 individuals divided into three groups: 45 probands, 23 affected relatives, and 30 unaffected relatives. All patients with RTH β (68 patients) were genetically confirmed as having *THRB* gene mutations. All participants provided informed consent to participate in this study. The study protocol was approved by the Ethics Committee of Kuma Hospital (No. 20200709-1) and was conducted in accordance with the principles of the Declaration of Helsinki.

Patients and methods

Participants

A total of 174 patients with probands suspected of having RTH β consulted Kuma Hospital and underwent *THRB* sequencing between July 2003 and May 2020. Among

Clinical characteristics and symptoms

The clinical characteristics of the patients were obtained from the medical records. Sixty-seven patients with RTH β were asked to answer a questionnaire and completed to describe their subjective symptoms. To assess concurrent atrial fibrillation, we evaluated the medical history and

Figure 1

Mutations of *THRB* in our study. The number of different families showing the same mutation is shown in brackets. Genetic variants that include one or more members with AITD are shown in bold.

Table 1 Types and characteristics of THR β mutations. Total numbers are indicated in parentheses.

Exon	TSH β mutation	Number of family	Male/female	AITD (positive/total examined members)	Data inconsistent with RTH β
7	A234V	1	1/1	1/2	Overt hypothyroidism (1)
7	A234D	1	2/2	2/4	–
7	A234T	1	1/1	1/2	–
7	Q235R	1	1/0	0/1	NA
7	W239R	1	0/1	1/1	–
7	Q241P	1	1/1	1/2	–
7	R243W	1	0/2	0/2	–
8	G251R	1	0/1	0/1	Subclinical thyrotoxicosis (1)
8	G261del	1	0/1	0/1	–
8	A268D	1	0/1	1/1	NA
8	I276N	1	0/1	0/1	–
9	M310V	1	0/1	0/1	–
9	M313T	2	2/0	2/2	Overt hypothyroidism (1)
9	R316H	1	0/1	1/1	Subclinical thyrotoxicosis (1)
9	A317T	3	0/3	0/2	NA
9	R320C	1	0/1	0/1	NA
9	Y321C	2	1/2	1/2	–
9	E333K	1	0/1	0/1	NA
9	R338L	2	1/4	3/5	–
9	R338Q	1	1/0	1/1	NA
9	R338W	2	0/2	1/2	–
9	K342I	1	2/3	5/5	Overt hypothyroidism (2) Overt thyrotoxicosis (2)
9	G347R	1	1/0	0/1	–
9	S350P	1	1/0	1/1	–
10	R383H	1	2/2	0/4	Euthyroidism (2)
10	R383S	1	0/1	0/1	–
10	R429Q	2	0/2	0/2	Subclinical thyrotoxicosis (1)
10	I431L	2	1/1	0/1	–
10	R438C	3	1/2	1/3	–
10	R438H	1	0/1	1/1	Overt hypothyroidism (1)
10	L450P	1	0/1	0/1	–
10	F451I	1	0/1	0/1	–
10	P453H	1	1/3	1/3	–
10	P453A	2	0/2	1/2	NA
10	I431fs	1	1/1	0/1	Subclinical thyrotoxicosis (1)
			(21/47)	(26/63)	

NA, not available.

checked pulse in all 68 patients at the initial examination. Further ECG examination was performed for 32 patients.

Laboratory and ultrasound tests

Laboratory results of 63 patients with RTH β and 25 unaffected relatives were available, while 5 patients with RTH β (A317T, Y321C, I431L, I431fs, and P453H) and 5 unaffected relatives were excluded because they were under the age of 15 (Supplementary Fig. 1, see section on [supplementary materials](#) given at the end of this article). Between July 2003 and December 2018, TSH, FT4, and FT3 levels were measured using chemiluminescent immunoassays (Abbott); reference ranges, TSH (0.3–4.9 μ IU/mL), FT4 (0.7–1.6 ng/dL), and FT3 (1.7–3.7 pg/

mL). After January 2019, electrochemiluminescence immunoassays (Roche Diagnostics); reference ranges, TSH (0.5–5.0 μ IU/mL), FT4 (0.9–1.7 ng/dL), and FT3 (2.3–4.0 pg/mL) were used.

Basal thyroid function tests were performed at least 1 month after patients who were misdiagnosed with Graves' disease discontinued anti-thyroid drugs. All participants except one unaffected relative were checked for thyroid autoantibodies (Supplementary Fig. 1) using methods described as follows. Before March 2008, four patients and one unaffected relative were evaluated by hemagglutination assay kits (microsomal hemagglutinin antibody (MCHA): Microsome test and thyroglobulin hemagglutinin antibody (TGHA): Thyroid test, Fuji Rebio Inc., Tokyo, Japan) and one patient was evaluated

Clinical Study	M Okazaki-Hada and others	Thyroid tests inconsistent with RTH β	186:1	76
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Table 2 Baseline characteristics of RTH β patients (45 probands). Data are presented as median (interquartile range, IQR).

Parameter	
Male/female	9/36
Age at diagnosis among probands (years)	36.0 (25.0–49.5)
0–10	6.7%
11–20	8.9%
21–30	22.2%
31–40	20.0%
41–50	22.2%
51–60	8.9%
>61	11.1%
Reasons for visit	
Goiter	22.2%
Palpitation	20.0%
Abnormal thyroid function*	57.8%

*Abnormal thyroid function found during the examination of symptoms that are not related to RTH, or during the health checkup.

using RIA for thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) (Cosmic Co, Hiroshima, Japan). After April 2008, TPOAb and TgAb were measured for 58 patients with RTH β and 23 unaffected relatives using an electrochemiluminescence immunoassay (Roche Diagnostics). For MCHA and TGHA, titers of 1:100 or more were regarded as positive. TPOAb ≥ 0.3 IU/mL and TgAb ≥ 0.3 IU/mL by RIA, and TPOAb ≥ 16.0 IU/mL and TgAb ≥ 28.0 IU/mL by electrochemiluminescence immunoassay were determined to be positive.

Data based on ultrasound examination were available in 56 patients with RTH β and in 21 unaffected relatives who were at least 15 years old (Supplementary Fig. 1). Thyroid volume was measured by ultrasound and was calculated using the equation as described previously (9). For evaluation of the malignant potential, we used the ultrasound classification system based on the shape and echo features of thyroid nodules as reported previously (10, 11) and measured the maximum diameter of nodule sizes.

The patients with AITD have satisfied the diagnostic criteria, that is, positive values for TPOAb and/or TgAb, with either hypoechoic and/or inhomogeneous pattern, in thyroid ultrasonography or lymphocytic infiltration in the thyroid gland with cytological examination.

Gene analysis

Genomic DNA was extracted from peripheral leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen). Exons 7–10 of the *THRB1* gene were amplified by PCR using a High Fidelity PCR Master (Roche Diagnostics). Conditions were as follows: initial denaturation for 10 min (94°C), followed

Table 3 Baseline characteristics of 68 RTH β patients (total; probands and relatives).

Parameter	
Male/female	21/47
Probands/relatives	45/23
Frequency of subjective symptoms	
Palpitation	38.8%
Goiter	34.3%
Sweating	23.9%
Hand tremor	13.4%
Anxiety	14.9%
Weight loss	11.9%
Misdiagnosed as Graves' disease	13.2%

by 35 cycles of denaturation for 1 min (94°C); annealing for 1 min (55°C); and elongation for 1 min (72°C), with a final elongation step at 72°C for 3 min. The four sets of forward and reverse primers were as follows: exon 7, 5'-CAG TAA GCC ATC TGT GCA TC-3' and 5'-GGC AAT AAC ACC AGT ATC CC-3'; exon 8, 5'-ACT GTA CAG GAT ATC AGT TC-3' and 5'-AGT ATT CCT GGA AAC TGA TG-3'; exon 9, 5'-TCA CAG AAG GTT ATT CCT ATT-3' and 5'-ACT CAA GTG ATT GGA ATT AG-3'; and exon 10, 5'-CTA AGA GGG AAG ACC CTA GA-3' and 5'-TTT CCC TCC CAA ATA ATC CC-3'. Direct sequencing of PCR products was performed using the Bigdye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and an automatic ABI 3130 sequencer (Applied Biosystems).

Statistical analysis

All analyses were performed using StatFlex version 6.0 software (Artech Co., Osaka, Japan). Values are presented as medians (interquartile range (IQR)). Categorical values were compared using a χ^2 test, and other values were analyzed using the Mann-Whitney *U*-test. Statistical significance was set at $P < 0.05$.

Results

Genetic testing revealed 35 heterozygous *THRB* gene mutations. All except two (p.R383H and p.R383S) were localized in three hot spot regions of the *THRB* described previously (2). They included 33 missense, 1 deletion, and 1 insertion mutation. The most prevalent mutations in this study were A317T and R438C, each harbored by three unrelated families (Fig. 1 and Table 1).

The clinical characteristics of the 45 probands with RTH β are shown in Table 2. The median age at diagnosis among probands was 36.0 (25.0–49.5) years, and over

40% of the patients were found to have RTH β for the first time at age, 40 years or older. Of the probands, 57.8% initially consulted our hospital for further investigation of RTH β due to abnormalities in their thyroid function test results. Collating the data of all patients with RTH β (Table 3), the questionnaire showed that palpitations and goiter were the main chief complaints and were present in 38.8 and 34.3% of the patients, respectively. Medical history and ECGs showed that 7.4% (5/68) of the patients had concurrent atrial fibrillation. Importantly, 13.2% (9/68) of the patients were misdiagnosed with Graves' disease at initial evaluation; consequently, nine patients were prescribed anti-thyroid drugs (six with thiamazole and two with propylthiouracil) and one patient who had undergone subtotal thyroidectomy at a hospital elsewhere was also prescribed thiamazole after recurrence of thyroid enlargement to 62 mL.

FT4 and FT3 levels were significantly higher in patients with RTH β compared to unaffected relatives (Table 4). Among RTH β patients, 42.1% of male patients and 40.9% of female patients had positive TPOAb and/or TgAb. The frequency of thyroid autoantibodies in male RTH β patients was significantly higher compared to unaffected male relatives ($P=0.019$). We also performed thyroid ultrasound and/or fine-needle aspirations for cytology in all RTH β

patients ($n=26$) and all unaffected relatives ($n=7$) who had positive thyroid autoantibodies. Hypoechoic and/or inhomogeneous pattern in ultrasonography was detected in all subjects but one. The remaining one had lymphocytic infiltration in cytological specimens in the thyroid. All 33 subjects showing positive thyroid autoantibodies had these typical findings of chronic thyroiditis defined as AITD. There were no significant differences in TSH levels (median (IQR), 2.8 (1.7–3.9) vs 2.0 (1.4–2.7) μ IU/mL, $P=0.052$) or ages (40.0 (27.3–59.3) vs 36.0 (28.5–48.5) years old, $P=0.46$) between RTH β patients with and without AITD. The median thyroid volume among RTH β patients was significantly larger compared to unaffected relatives ($P=0.0033$ in male and 0.0072 in female, respectively). While 34.3% presented with a subjective symptom of goiter (Table 3), the ultrasound data showed that twice as many patients (73.2%) had goiter with a thyroid volume ≥ 20 mL. While nodules with solid components were detected in 42.9% of the RTH β patients, malignancy was not suspected in any of them upon ultrasound evaluation and fine-needle aspiration.

We evaluated the sequential thyroid function in 44 RTH β patients who consulted our hospital more than twice (Supplementary Fig. 1). Among them, 13 patients (29.5%), 8 with AITD and 5 without AITD, temporarily showed data

Table 4 Laboratory and ultrasound data (RTH β vs unaffected relatives, data age >15 years old). Data are presented as median (IQR). AITD pattern: hypoechoic and/or inhomogeneous pattern.

	RTH β ($n=63$)	unaffected relatives ($n=25$)	<i>P</i> value
Male/female	19/44	6/19	0.56
TSH (μ IU/mL)	2.2 (1.5–3.2)	1.9 (1.0–2.6)	0.15
FT4 (ng/dL)	1.9 (1.8–2.2)	1.1 (1.0–1.3)	<0.0001
FT3 (pg/mL)	4.8 (4.2–5.6)	2.9 (2.8–3.1)	<0.0001
Antibody positivity			
Male	8/19 (42.1%)	0/6 (0.0%)	0.019
TPOAb positive	6/19 (31.6%)	0/6 (0.0%)	0.050
TgAb positive	7/19 (36.8%)	0/6 (0.0%)	0.031
Female	18/44 (40.9%)	7/18 (38.9%)	0.88
TPOAb positive	14/44 (31.8%) [#]	5/18 (27.8%)	0.75
TgAb positive	17/44 (38.6%) ^{##}	7/18 (38.9%)	0.99
Ultrasound findings			
Thyroid volume (mL)			
Male	31.9 (24.6–43.4)	13.3 (11.6–19.4)	0.0033
Female	26.4 (18.1–37.2)	14.4 (10.2–19.8)	0.0072
Presence of nodules	24/56 (42.9%)	6/21 (28.6%)	0.25
Nodule size (mm)			
<5	3/56 (5.4%)	2/21 (9.5%)	
5–10	11/56 (19.6%)	3/21 (14.3%)	
10–20	5/56 (8.9%)	1/21 (4.8%)	
>20	5/56 (8.9%)	0/21 (0.0%)	
AITD pattern in antibody positive patients	25/26	7/7	0.49

[#]Including two patients with positive MCHA (microsomal hemagglutinin antibody); ^{##}Including two patients with positive TGHA (thyroglobulin hemagglutinin antibody).

Clinical Study	M Okazaki-Hada and others	Thyroid tests inconsistent with RTH β	186:1	78
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Table 5 Sequential thyroid function tests available in RTH β patients with and without AITD. Data are presented as median (IQR).

	AITD (+)	AITD (–)	P value
Number	19	25	
Age (years old)	39.0 (25.0–57.0)	36.0 (28.8–48.5)	0.85
Observation period (months)	73.0 (24.0–127.0)	39.0 (16.0–108.3)	0.34
Thyroid function tests inconsistent with RTH β (n (%))	8 (42.1%)	5 (20%)	0.11
Overt hypothyroidism	5	0	
Euthyroidism	0	2	
Subclinical thyrotoxicosis	1	3	
Overt thyrotoxicosis	2	0	

inconsistent with RTH β during the observation period (Table 5). Serial changes of data in each individual had been evaluated under the same assay condition. Although its prevalence between RTH β patients with and without AITD was not different, overt hypothyroidism (Table 6 and Supplementary Fig. 2) with elevated TSH and low FT4 levels and overt thyrotoxicosis (Table 7 and Supplementary Fig. 3) with low TSH and high FT4 levels were detected only among seven patients with AITD. These patients complained of symptoms associated with fluctuating thyroid function and required treatment. Thyroid function test results consistent with RTH β were detected at various time points (Tables 6, 7, and Supplementary Figs 2, 3). Two patients having the R383H mutation temporarily showed normal thyroid hormone and TSH levels without AITD (Tables 1 and 5). There were no over-represented mutations in the group with data inconsistent with RTH β (Table 1).

Discussion

This study describes the characteristics of 68 Japanese patients with RTH β in a single institute specializing in thyroid care. We found that RTH β patients, especially men, showed a higher frequency of AITD. More than 20% of patients with RTH β temporarily showed thyroid tests inconsistent with RTH β during their follow-up, and patients with AITD and specific mutations of *THRB* were prone to display such findings.

The presence of thyroid autoimmunity is generally more common in women; however, there was no difference in sex regarding the frequency of AITD in RTH β patients in our study. Moreover, the frequency of AITD in male RTH β patients was significantly higher compared to unaffected relatives; however, this difference was not found in the female patients (Table 4). This result was consistent with

Table 6 Cases of RTH β showing overt hypothyroidism due to chronic thyroiditis. Laboratory data at the start of levothyroxine (LT4) treatment is presented. TPOAb and TgAb was estimated by electrochemiluminescence immunoassay for all cases except Case 4, where hemagglutination assay, MCHA and TGHA was used.

Case detail/ Case	Gender	TSH (μ IU/mL)	FT4 (ng/dL)	FT3 (pg/mL)	TPOAb (IU/mL)	TgAb (IU/mL)	THRB mutation
Initially diagnosed with chronic thyroiditis. After LT4 replacement, thyroid function began to show data consistent with RTH β . Further evaluation revealed a mutation in THRB.							
Case 1	F	200*	0.7	NT	366.0	144.5	K342I
Case 2	F	97.8	0.6	NT	509.4	316.9	K342I
Case 3	F	130.3	0.7	2.4	19.4	836.3	A234V
Reference range for cases 1, 2 and 3		0.3–4.0	0.8–2.1	2.2–5.6	<16.0	< 28.0	
Initial thyroid function showed data consistent with RTH β . Further evaluation revealed a mutation in THRB. During the follow up, TSH level began to be elevated.							
Case 4	F	72.9	0.9	4.2	1:1600	1:100	R438H
Reference range		0.3–4.0	0.8–2.1	2.2–5.6	<1:100	<1:100	
Case 5	M	>100	0.3	2.0	447.5	247.6	M313T
Reference range		0.3–4.9	0.7–1.6	1.7–3.7	<16.0	< 28.0	

F, female; M, male; NT, not tested.

the study of Barkoff *et al.* (6). In addition, the prevalence of AITD in Japanese patients with RTH β in this study (more than 40%) was higher compared to previous studies (~25%) (4, 6, 7). Japanese individuals are known to have a higher iodine intake than individuals from other countries (12). Although the relationship between iodine intake and the occurrence of AITD is undetermined, several studies have shown a direct relationship between iodine intake and AITD (13, 14). The mechanism of increased AITD in patients with RTH β has not been elucidated. Gavin *et al.* have suggested that chronic TSH stimulation in RTH β patients activates intrathyroidal lymphocytes to produce pro-inflammatory cytokines such as TNF- α , leading to thyroid cell destruction causing AITD (15); however, this hypothesis remains controversial (6).

To the best of our knowledge, no studies have evaluated the sequential thyroid functions and ultrasound findings of a large number of patients with RTH β . Although RTH β patients are known to exhibit discrepant thyroid test results, 29.5% of patients in this study temporarily showed data inconsistent with RTH β . Among these, 88% (7/8) of RTH β patients with AITD developed overt hypothyroidism or thyrotoxicosis and required treatment (Tables 1, 5, 6, 7, and Supplementary Figs 2, 3). In this study, four of five members in a family with RTH β caused by K432I and concurrent AITD presented with overt thyrotoxicosis or overt hypothyroidism (Tables 1, 6 and 7). In the past, several RTH β cases have been reported showing changes in thyroid function due to chronic thyroiditis (16, 17, 18), Graves' disease (19, 20, 21, 22, 23, 24, 25, 26), and painless thyroiditis (27, 28), which required treatment.

In contrast, RTH β patients without AITD showed only mild and temporary changes of thyroid function test results atypical of RTH β (Tables 1 and 5). Among them, two RTH β cases with the R383H mutation and without AITD in this study temporarily showed normal thyroid hormone levels with TSH levels in the normal range. Although R383 is located outside of the three hot spot regions, the R383H mutation has been previously reported to cause a mild form of RTH β with small goiter, tachycardia, slightly elevated FT4, and normal TSH level (29). *In vitro* analysis showed that the R383H mutation had a T3-binding affinity of 70% that of WT and little dominant negative effect, suggesting the importance of the region for dimerization of the receptor (29, 30). Similarly, several other cases of RTH β (P247L, E311K, R316C, G385E, and R429W), in addition to R383H, presented occasionally normal thyroid hormone levels (31, 32, 33, 34, 35). Two mutants (P247L and R429W) manifest either mild impairment of T3-binding or a weak dominant-negative effect (30, 31, 32). Structurally, the E311K mutant

Table 7 Cases of RTH β showing overt thyrotoxicosis.

Case	Detail	Gender	TSH (μ U/mL)	FT4 (ng/dL)	FT3 (pg/mL)	TPOAb (IU/mL)	TgAb (IU/mL)	TRAb (IU/mL)	RAIU (%)	THRB mutation
1	Initial thyroid function test showed overt thyrotoxicosis. From his family history, survey of RTH β revealed a mutation in THRB. (RTH β coexisted with Graves' disease)	M	0.029	2.1	7.5	>600	377.6	3.7	9.9*	K342I
2	Initial thyroid function test showed data compatible to RTH β and further evaluation revealed a mutation in THRB. During the follow up, the laboratory data began to show overt thyrotoxicosis. (RTH β coexisted with painless thyroiditis) Reference Range	F	0.006	7.7	28.5	58.0	610.9	<0.8	2.8**	K342I
			0.5-5.0	0.9-1.7	2.3-4.0	<16.0	<28.0	<2.0		

Reference range at *at three hours: 5-15%, ** at 24 hours: 10-40%. RAIU, radioactive iodine uptake; F, female; M, male

leads to a loss of hydrogen bonds between R383 (33) and the G385E mutant, which is localized adjacent to R383 within the dimerization region (35). Although genotype–phenotype correlation in patients with RTH β is still controversial (36), we must be aware of fluctuations in thyroid function among patients with RTH β due to both concurrent AITD and mutant receptor properties.

Ultrasound examinations revealed a high frequency of thyroid nodules in RTH β patients, but no thyroid cancer. Indeed, only a few cases have been reported to have thyroid cancer concurrent with RTH β (37, 38, 39). On the other hand, a mutant knock-in mouse with a targeted potent dominant negative TR β mutant, which is identical in a patient with RTH β (40), spontaneously developed thyroid cancer when homozygous, but not when heterozygous (41). Apart from the dominant-negative effect for TR β regulation, multiple signaling pathways, including non-genomic mechanisms, are induced by the mutation of the two alleles of the *THRB* gene, consequently leading to thyroid carcinogenesis (42, 43). Both case studies and experimental findings in previous reports indicate that although elevated TSH levels may promote thyroid cell proliferation, patients with a heterozygous *THRB* mutation have no obvious risk for the development of thyroid cancer.

This study has some limitations. This was a retrospective study with varied follow-up periods and a relatively small number of participants in the unaffected relatives group. The median age at diagnosis among probands was 36.0 years, possibly because our hospital has no department of pediatrics; otherwise, Japanese general practitioners are unaware of RTH β . In addition, 13.2% were misdiagnosed to have Graves' disease at initial evaluation; however, all misdiagnoses were corrected to RTH β before 2011. The American Thyroid Association guidelines (44) and the diagnostic criteria for RTH β published by the Japan Thyroid Association (available at: www.japanthyroid.jp/en/clinical.html accessed May 23, 2021) have led to an increased awareness of RTH β resulting in a decrease in the frequency of its misdiagnosis.

In conclusion, a higher frequency of AITD and thyroid test results fluctuations in RTH β patients may obscure the presence of RTH β during follow-up. Suspicion of RTH β should prompt physicians to consider repeat laboratory tests at different time periods and performing genetic testing to avoid misdiagnosis and inappropriate treatment.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-21-0584>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

M O-H and E N designed the study. M O-H extracted the data and performed the statistical analysis. M O-H and E N wrote the manuscript. M H and T K contributed to the patients care and acquisition of data. M I, S F, M N, T A, and A M critically reviewed the article. All authors discussed the results of the study and approved the final manuscript.

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