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 (RTHβ) is an inherited syndrome caused by mutations in the thyroid hormone receptor β (THRB) gene. Patients with RTHβ typically have elevated thyroid hormone levels with non-suppressed serum TSH. We aimed to elucidate the clinical, laboratory, and imaging findings of RTHβ 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 RTHβ patients (45 probands and 23 relatives) and 30 unaffected relatives in Kuma Hospital.

Results

Genetic testing revealed 35 heterozygous THRB gene mutations. Among all RTHβ 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%; eight [42.1%] with AITD and five [20%] without) temporarily showed thyroid function test results inconsistent with RTHβ. 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 RTHβ patients was significantly higher compared to unaffected relatives. More than 20% of RTHβ patients temporarily showed laboratory findings atypical of RTHβ 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.
Introduction

Resistance to thyroid hormone beta (RTHβ) 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 RTHβ, with more than 4000 patients belonging to over 600 families (2). In general, patients with RTHβ are identified by discrepant thyroid function test results, elevated serum levels of free T4 (FT4) and free T3 (FT3) with non-suppressed TSH. Most studies involving a large number of patients with RTHβ originate from North America and Europe (3-5). However, no studies involving a large series of patients with RTHβ have been conducted in Asian countries including Japan.

Barkoff et al. reported that 23.3% of patients with RTHβ had a comorbid autoimmune thyroid disease (AITD). They described that individuals with RTHβ 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 RTHβ in Spain and South America, respectively. However, these studies did not investigate the increase in thyroid autoantibodies in patients with RTHβ 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 RTHβ.

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

**Materials and Methods**

**Participants**

A total of 174 patients with probands suspected of having RTHβ consulted Kuma Hospital and underwent THRB sequencing between July 2003 and May 2020. Among them, 45 patients were confirmed to have THRB mutations. Relatives of these patients underwent gene analysis to determine if any 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 RTHβ (68 patients) were genetically confirmed as having \textit{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 conducted in accordance with the principles of the Declaration of Helsinki.

\textit{Clinical characteristics and symptoms}

The clinical characteristics of the patients were obtained from the medical records. Sixty-seven patients with RTHβ were asked to answer a questionnaire and completed to describe their subjective symptoms. To assess concurrent atrial fibrillation, we evaluated medical history and checked pulse in all 68 patients at initial examination. Further electrocardiogram examination was performed for 32 patients.

\textit{Laboratory and ultrasound tests}

Laboratory results of 63 patients with RTHβ and 25 unaffected relatives were available, while five patients with RTHβ (A317T, Y321C, I431L, I431fs, and P453H) and five unaffected relatives were excluded because they were under the age of 15 (supplementary Figure 1). Between July 2003 and December 2018, TSH, FT4, and FT3...
levels were measured using chemiluminescent immunoassays (Abbott, Abbott Park, Illinois, USA); reference ranges, TSH (0.3-4.9 μU/mL), FT4 (0.7-1.6 ng/dL), FT3 (1.7-3.7 pg/mL) after January 2019, electrochemiluminescence immunoassays (Roche Diagnostics, Mannheim, Germany); reference ranges, TSH (0.5-5.0 μU/mL), FT4 (0.9-1.7 ng/dL), FT3 (2.3-4.0 pg/mL) were used.

Basal thyroid function tests were performed at least one month after patients who were misdiagnosed with Graves’ disease discontinued anti-thyroid drugs. All participants except one unaffected relative were checked for thyroid autoantibodies (Table 4, supplementary Figure 1) using methods described as follows. Before March 2008, four patients and one unaffected relative were evaluated by hemagglutination assay kits (MCHA: Microsome test and TGHA: Thyroid test, Fuji Rebio Inc., Tokyo, Japan) and one patient was evaluated by radioimmunoassay 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 RTHβ and 23 unaffected relatives using an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). For MCHA and TGHA, titers of 1:100 or more
were regarded as positive. TPOAb $\geq 0.3$ IU/mL and TgAb $\geq 0.3$ IU/mL by radioimmunoassay, and TPOAb $\geq 16.0$ IU/mL and TgAb $\geq 28.0$ IU/mL by electrochemiluminescence immunoassay were determined to be positive.

Data based on ultrasound examination were available in 56 patients with 
RTHβ and in 21 unaffected relatives who were at least 15 years old (Table 4, 
 supplementary Figure 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: 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, Hilden, Germany). Exons 7–10 of the THRBI gene were amplified by polymerase chain reaction (PCR) using a High Fidelity PCR Master (Roche Diagnostics, Mannheim, Germany). Conditions were as follows: initial denaturation for 10 min (94 °C), followed 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 TCA AAT AG-3’. Direct sequencing of PCR products was performed using the Bigdye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, Massachusetts, USA) and an automatic ABI 3130 sequencer (Applied Biosystems, Waltham, Massachusetts, USA).

Statistical analysis
All analyses were performed using StatFlex version 6.0 software (Artech Co., Osaka, Japan). Values are presented as medians (interquartile range). Categorical values were compared using a χ² test, and the 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, p.R383S) were localized in the three hot spot regions of the *THRB* described previously (2). They included 33 missense, one deletion, and one insertion mutation. The most prevalent mutations in this study were A317T and R438C, each harbored by three unrelated families (Table 1 and Figure 1).

The clinical characteristics of the 45 probands with RTHβ 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 RTHβ for the first time at age 40 years or older. Of the probands, 57.8% initially consulted our hospital for further investigation of RTHβ due to abnormalities in their thyroid function test results. Collating the data of all
patients with RTHβ (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 electrocardiograms showed that 7.4% (5 of 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 (6 with thiamazole and 2 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 RTHβ compared to unaffected relatives (Table 4). Among RTHβ 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 RTHβ 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 RTHβ 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 remain 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] μIU/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 RTHβ patients with and without AITD. The median thyroid volume among RTHβ 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 \( \geq 20 \) mL. While nodules with solid components were detected in 42.9% of the RTHβ patients, malignancy was not suspected in any of them upon ultrasound evaluation and fine needle aspiration.

We evaluated the sequential thyroid function in 44 RTHβ patients who consulted our hospital more than twice (supplementary Figure 1). Among them, 13 patients (29.5%), eight with AITD and five without AITD, temporarily showed data inconsistent with RTHβ 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 RTHβ patients with and without AITD was not different, overt hypothyroidism (Table 6 and supplementary Figure 2) with elevated TSH and low FT4 levels and overt thyrotoxicosis (Table 7 and supplementary Figure 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 RTHβ were detected at various time points (Tables 6, 7 and supplementary Figures 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 RTHβ (Table 1).

Discussion

This study describes the characteristics of 68 Japanese patients with RTHβ in a single institute specializing in thyroid care. We found that RTHβ patients, especially men, showed a higher frequency of AITD. More than 20% of patients with RTHβ
temporarily showed thyroid tests inconsistent with RTHβ during their follow-up, and
patients with AITD and specific mutations of THRβ 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 RTHβ
patients in our study. Moreover, the frequency of AITD in male RTHβ 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 the study of
Barkoff et al (6). In addition, the prevalence of AITD in Japanese patients with RTHβ 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 RTHβ has not been elucidated. Gavin et al. have suggested that chronic
TSH stimulation in RTHβ patients activates intra-thyroidal 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 RTHβ. Although RTHβ patients are known to exhibit discrepant thyroid test results, 29.5% of patients in this study temporarily showed data inconsistent with RTHβ. Among these, 88% (7/8) of RTHβ patients with AITD developed overt hypothyroidism or thyrotoxicosis and required treatment (Tables 1, 5-7 and supplementary Figures 2, 3). In this study, four of five members in a family with RTHβ caused by K432I and concurrent AITD presented with overt thyrotoxicosis or overt hypothyroidism (Tables 1, 6, and 7). In the past, several RTHβ cases have been reported showing changes in thyroid function due to chronic thyroiditis (16-18), Graves’ disease (19-26), and painless thyroiditis (27, 28), which required treatment.

In contrast, RTHβ patients without AITD showed only mild and temporary changes of thyroid function test results atypical of RTHβ (Tables 1 and 5). Among them, two RTHβ 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 RTHβ 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 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 RTHβ (P247L, E311K, R316C, G385E, and R429W), in addition to R383H, presented occasionally normal thyroid hormone levels (31-35). Two mutants (P247L and R429W) manifest either mild impairment of T3-binding or a weak dominant negative effect (30-32). Structurally, the E311K mutant 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 RTHβ is still controversial (36), we must be aware of fluctuations in thyroid function among patients with RTHβ due to both concurrent AITD and mutant receptor properties.

Ultrasound examinations revealed a high frequency of thyroid nodules in
RTHβ patients, but no thyroid cancer. Indeed, only a few cases have been reported to have thyroid cancer concurrent with RTHβ (37-39). On the other hand, a mutant knock-in mouse with a targeted potent dominant negative TRβ mutant, which is identical in a patient with RTHβ (40), spontaneously developed thyroid cancer when homozygous, but not when heterozygous (41). Apart from the dominant negative effect for TR 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 RTHβ. In addition, 13.2% were misdiagnosed to have
Graves’ disease at initial evaluation; however, all misdiagnoses were corrected to RTHβ before 2011. The American Thyroid Association guidelines (44) and the diagnostic criteria for RTHβ 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 RTHβ resulting in a decrease in the frequency of its misdiagnosis.

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

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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**Figure Legends**

Figure 1. Mutations of THRBB in our study

The number of different families showing the same mutation are shown in brackets.

Genetic variants that include one or more members with AITD are shown in bold.
Supplementary Figure Legends

Supplementary Figure 1. Flowchart for this study enrollment in patients with RTHβ and unaffected relatives

Numbers available for each examination and excluded from the evaluation were indicated in parentheses. The related Tables are also shown in the figure.

Supplementary Figure 2. Serial thyroid function data of five patients with RTHβ showing overt hypothyroidism due to chronic thyroiditis in Table 6

Arrows indicate the onset of hypothyroidism. Each vertical bar indicates a reference range.

Supplementary Figure 3. Serial thyroid function data of two patients with RTHβ showing overt thyrotoxicosis in Table 7

Arrows indicate the onset of thyrotoxicosis. Each vertical bar indicates a reference range.
Table 1. Types and characteristics of THRB mutations

| Exon | TSHB mutation | Number of family | Male /Female | AITD (positive/ total examined members) | Data inconsistent with RTHβ |
|------|---------------|------------------|--------------|----------------------------------------|-----------------------------|
| 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

Total numbers are indicated in parentheses.
Table 2. Baseline characteristics of RTHβ patients (45 probands)

| 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%         |        |

Data are presented as median (interquartile range, IQR).
*abnormal thyroid function found during the examination of symptoms that are not related to RTH, or during the health checkup.
Table 3. Baseline characteristics of 68 RTHβ patients (total; probands and relatives)

| Parameter                  | Male / Female | Probands/Relatives |
|----------------------------|---------------|--------------------|
| Male / Female              | 21 / 47       | 45 / 23            |
| Misdiagnosed as Graves’ disease | 13.2%         |                    |
| Frequency of subjective symptoms |             |                    |
| Palpitation                | 38.8%         |                    |
| Goiter                     | 34.3%         |                    |
| Sweating                   | 23.9%         |                    |
| Hand tremor                | 13.4%         |                    |
| Anxiety                    | 14.9%         |                    |
| Weight loss                | 11.9%         |                    |
Table 4. Laboratory and ultrasound data (RTHβ vs unaffected relatives, data age >15 years old)

|                     | RTHβ (n=63) | unaffected relatives (n=25) | P value |
|---------------------|-------------|----------------------------|---------|
| Male / Female       | 19/44       | 6/19                       | 0.56    |
| TSH (μU/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 | Female | Male | Female | P value |
|---------------------|------|--------|------|--------|---------|
| Male                | 8/19 (42.1%) | 18/44 (40.9%) | 0/6 (0.0%) | 0/18 (0.0%) | 0.019 |
| TPOAb positive      | 6/19 (31.6%) | 14/44 (31.8%) | 0/6 (0.0%) | 5/18 (27.8%) | 0.75 |
| TgAb positive       | 7/19 (36.8%) | 17/44 (39.6%) | 0/6 (0.0%) | 7/18 (38.9%) | 0.99 |

Ultrasound findings

|                     | Male | Female | P value |
|---------------------|------|--------|---------|
| Thyroid volume (mL) | 31.9 (24.6-43.4) | 26.4 (18.1-37.2) | 0.0033 |
| Presence of nodules | 24/56 (42.9%) | 22/56 (39.3%) | 0.25 |
| Nodule size         | < 5mm | 5/56 (8.9%) | 2/21 (9.5%) | 0.25 |
|                     | 5-10mm | 11/56 (19.6%) | 3/21 (14.3%) | 0.25 |
|                     | 10-20mm | 5/56 (8.9%) | 1/21 (4.8%) | 0.25 |
|                     | > 20mm | 5/56 (8.9%) | 0/21 (0.0%) | 0.25 |
| AITD pattern in antibody positive patients | 25/26 | 7/7 | 0.49 |

Data are presented as median (IQR).

# including two patients with positive MCHA (Microsomal hemagglutinin antibody)

## including two patients with positive TGHA (Thyroglobulin hemagglutinin antibody)

AITD pattern: hypoechoic and/or inhomogeneous pattern
Table 5. Sequential thyroid function tests available in RTHβ patients with and without AITD

|                                | 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 RTHβ [number (%)] | 8 (42.1%) | 5 (20%) | 0.11 |
| overt hypothyroidism           | 5        | 0        |         |
| euthyroidism                   | 0        | 2        |         |
| subclinical thyrotoxicosis     | 1        | 3        |         |
| overt thyrotoxicosis           | 2        | 0        |         |

Data are presented as median (IQR).
Table 6. Cases of RTHβ showing overt hypothyroidism due to chronic thyroiditis

| case | detail                                                                 | gender | TSH (μIU/mL) | FT4 (ng/dL) | FT3 (pg/mL) | TPOAb | TgAb | THRβ mutation |
|------|------------------------------------------------------------------------|--------|--------------|-------------|-------------|-------|------|---------------|
| 1    | Initially diagnosed with chronic thyroiditis. After LT4 replacement, thyroid function began to show data consistent with RTHβ. Further evaluation revealed a mutation in THRβ. | F      | 200*         | 0.7*        | NT          | 366.0# | 144.5# | K342I         |
| 2    | Initially diagnosed with chronic thyroiditis. After LT4 replacement, thyroid function began to show data consistent with RTHβ. Further evaluation revealed a mutation in THRβ. | F      | 97.8*        | 0.6*        | NT          | 509.4# | 316.9# | K342I         |
| 3    | Initially diagnosed with chronic thyroiditis. After LT4 replacement, thyroid function began to show data consistent with RTHβ. Further evaluation revealed a mutation in THRβ. | F      | 130.3*       | 0.7*        | 2.4*        | 19.4#  | 836.3# | A234V         |
| 4    | Initial thyroid function showed data consistent with RTHβ. Further evaluation revealed a mutation in THRβ. During the follow up, TSH level began to be elevated. | F      | 72.9*        | 0.9*        | 4.2*        | 1:1600## | 1:100## | R438H         |
| 5    | Initial thyroid function showed data consistent with RTHβ. Further evaluation revealed a mutation in THRβ. During the follow up, TSH level began to be elevated. | M      | > 100**      | 0.3**       | 2.0**       | 447.5** | 247.6** | M313T         |

Laboratory data when starting the levothyroxine (LT4) treatment

*reference ranges: TSH (0.3-4.0 μU/mL), FT4 (0.8-2.1 ng/dL), FT3 (2.2-5.6 pg/mL)

**reference ranges: TSH (0.3-4.9 μU/mL), FT4 (0.7-1.6 ng/dL), FT3 (1.7-3.7 pg/mL)

# electrochemiluminescence immunoassay, TPOAb; reference range < 16.0 IU/mL, TgAb; reference range < 28.0 IU/mL

## hemagglutination assay, MCHA and TGHA; reference range less than 1:100

F, female; M, male; NT, not tested
Table 7. Cases of RTHβ showing overt thyrotoxicosis

| case | detail                                                                 | gender | TSH (μIU/mL) | FT4 (ng/dL) | FT3 (pg/mL) | TPOAb (IU/mL) | TgAb (IU/mL) | TRAb (IU/L) | RAIU (%) | mutation |
|------|------------------------------------------------------------------------|--------|--------------|-------------|-------------|---------------|--------------|-------------|----------|----------|
| 1    | Initial thyroid function test showed overt thyrotoxicosis. From his   | M      | 0.029        | 2.1         | 7.5         | >600          | 377.6        | 3.7         | 9.9*     | K342I    |
|      | family history, survey of RTHβ revealed a mutation in THRB. (RTHβ     |        |              |             |             |               |              |             |          |          |
|      | coexisted with Graves’ disease)                                        |        |              |             |             |               |              |             |          |          |
| 2    | Initial thyroid function test showed data compatible to RTHβ and      | F      | 0.006        | 7.7         | 28.5        | 58.0          | 610.9        | <0.8        | 2.8**    | K342I    |
|      | further evaluation revealed a mutation in THRB. During the follow up,|        |              |             |             |               |              |             |          |          |
|      | the laboratory data began to show overt thyrotoxicosis. (RTHβ         |        |              |             |             |               |              |             |          |          |
|      | coexisted with painless thyroiditis)                                   |        |              |             |             |               |              |             |          |          |

Reference ranges: TSH (0.5-5.0 μU/mL), FT4 (0.9-1.7 ng/dL), FT3 (2.3-4.0 pg/mL), TRAb (< 2.0 IU/L), TPOAb < 16.0 IU/mL, TgAb < 28.0 IU/mL

Reference ranges: *radioactive iodine uptake (RAIU) at three hours, 5-15%; ** RAIU at 24 hours, 10-40%

F, female; M, male
Figure 1

DNA-binding  Hinge  T3-Binding

Exon7  Exon8  Exon9  Exon10

Hot Spot 1  426-469
- p. R429Q(2)
- p. R431L(2)
- p. R438C(3)
- p. R438H
- p. L450P
- p. F451H
- p. P453H
- p. P453A(2)
- p. I431L

Hot Spot 2  302-357
- p. M311V
- p. M313T(2)
- p. R316H
- p. A317T(3)
- p. R320C
- p. Y321C(2)
- p. E333K
- p. R338H(2)
- p. R338Q
- p. R338W(2)
- p. K342I
- p. G347R
- p. S350P

Hot Spot 3  234-282
- p. A234V
- p. A234D
- p. A234T
- p. Q235R
- p. W239R
- p. Q241P
- p. R243W
- p. G251R
- p. G261del
- p. A268D
- p. L276N

338x190mm (300 x 300 DPI)
Supplementary Figure 1

RTHji (n=66)
  • Probands (n=45)  <Table 3>
  • Relatives (n=23)
    - Younger patients (n=5)

>15 years old (n=63)
  <Table 4>
  • TgAb, TPOAb examination (n=63)
  • US examination (n=56)
    - No available follow-up data (n=19)

Follow-up of thyroid function (n=44)
  • With AITD (n=19)
  • Without AITD (n=25)

Unaffected relatives (n=30)
  • Younger subjects (n=5)

>15 years old (n=25)
  <Table 4>
  • TgAb, TPOAb examination (n=24)
  • US examination (n=21)
Supplementary Figure 2

Case 1

Case 2

Case 3

Case 4

Case 5

TSH (mIU/L)
0 25 50 75 100

T3u (μg/dL)
0 1 2 3

T4u (μg/dL)
0 1 2 3

FT3 (ng/mL)

FT4 (ng/mL)

338x225mm (300 x 300 DPI)
Supplementary Figure 3

Case 1

Case 2

338x225mm (300 x 300 DPI)