A family of RTHβ with p.R316C mutation presenting occasional syndrome of inappropriate secretion of TSH

Yohei Ueda1), Tetsuya Tagami1), Tatsuya Tamanaha1), Maiko Kakita1), Kanako Tanase-Nakao1), Kazutaka Namba1), Takeshi Usui2), Mitsuhide Naruse2) and Akira Shimatsu2)

1) Department of Endocrinology and Metabolism, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Japan
2) Clinical Research Institute for Endocrine and Metabolic Diseases, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Japan

Abstract. The syndrome of inappropriate secretion of thyrotropin (SITSH) is a hallmark of resistance to thyroid hormone (RTH) due to mutations in the β isoform of the thyroid hormone receptor (TRβ). Here, we report on a family of RTH due to a TRβ mutation (RTHβ) and presenting occasional SITSH. The proband was a 16 year-old girl with a goiter, detected at a school physical examination. She was initially diagnosed as having euthyroid Hashimoto thyroiditis because her thyroid function was normal with a positive anti-thyroglobulin antibody. Follow-up examinations resulted in mild SITSH on some occasions and euthyroid on the other occasions. A magnetic resonance imaging (MRI) revealed a normal pituitary gland. Because her mother also had mild SITSH, genetic analysis was performed and revealed a heterozygous point mutation in TRβ (p.R316C). Previously, the p.R316C had only been found in severe RTH cases with homozygous mutations or with an ectopic thyroid. Her mother with a heterozygous mutation showed variable RTH phenotype on T3 suppression testing. In conclusion, the prevalence of RTHβ might be underestimated and occasional SITSH could also suggest RTHβ. TRβ gene mutation is not always correlated with the RTH phenotype.

Key words: SITSH, RTH, RTHβ, TRβ, p.R316C
As a differential diagnosis with a TSH producing pituitary tumor, magnetic resonance imaging (MRI) was performed and revealed normal pituitary.

We investigated the thyroid function of her family members and found mild SI-TSH also in the proband’s mother. She is also short of 144 cm height. Her thyroid hormones were fluctuated and showed occasional elevation of thyroid hormones with normal levels of TSH similar to the proband (Fig. 1B). Both of her TgAb and anti-thyroid peroxidase antibodies (TPOAb) were negative. She was admitted to our hospital for hypertension, diabetes mellitus, dyslipidemia, and osteoporosis. Her blood pressure was 176/112 mmHg and pulse rate was regular but 117 per minute. The levels of CK, TC, triglyceride and fasting plasma glucose were 104 IU/L, 266 mg/dL, 500 mg/dL and 221 mg/dL, respectively. Her bone mineral density (BMD) was 0.649 g/cm² (64% of the young adult mean [YAM]) in lumbar and 0.482 g/cm² (61% of the YAM) in femur. Her father (52 years old and 170cm in height) had HT but his thyroid hormones were within the normal range at the several points of time. Her two brothers (18 years old, 166cm and 23 years old, 156cm) were also euthyroid and their anti-thyroid antibodies were negative.

Laboratory evaluation

The levels of ferritin and other biochemical parameters were measured as routine examination. Sex hormone binding globulin (SHBG) was measured with a two-site directed chemiluminescence immunoassay (two-site CLIA) (LSI Medience, Corp., Tokyo, Japan). Serum concentrations of FT3, FT4 and TSH were

**Patients and Methods**

**Patients**

A 16 year-old girl visited our hospital with a complaint of goiter detected at a school physical examination. She was short of 149 cm height and her weight was 38 kg. Physical examination revealed a blood pressure of 107/63 mmHg and a regular pulse of 75 per minute. She was initially euthyroid; free triiodothyronine (FT3; 3.5pg/mL), free thyroxine (FT4; 1.6 ng/dL) and TSH (1.74 mU/L) were within the normal range, but anti-thyroglobulin antibody (TgAb) was weakly positive. The level of creatine kinase (CK) was 56 IU/L and total cholesterol (TC) was 165 mg/dL. Under the diagnosis of euthyroid Hashimoto thyroiditis (HT), we followed up her thyroid function one year later and revealed that FT4 was slightly elevated with a normal TSH level. After that, we followed up her thyroid function periodically and revealed that her thyroid hormones were slightly elevated on some occasions and within normal range on the other occasions, but TSH levels were constantly within normal range (Fig. 1A). As a differential diagnosis with a TSH producing pituitary tumor, magnetic resonance imaging (MRI) was performed and revealed normal pituitary.

We investigated the thyroid function of her family members and found mild SI-TSH also in the proband’s mother. She is also short of 144 cm height. Her thyroid hormones were fluctuated and showed occasional elevation of thyroid hormones with normal levels of TSH similar to the proband (Fig. 1B). Both of her TgAb and anti-thyroid peroxidase antibodies (TPOAb) were negative. She was admitted to our hospital for hypertension, diabetes mellitus, dyslipidemia, and osteoporosis. Her blood pressure was 176/112 mmHg and pulse rate was regular but 117 per minute. The levels of CK, TC, triglyceride and fasting plasma glucose were 104 IU/L, 266 mg/dL, 500 mg/dL and 221 mg/dL, respectively. Her bone mineral density (BMD) was 0.649 g/cm² (64% of the young adult mean [YAM]) in lumbar and 0.482 g/cm² (61% of the YAM) in femur. Her father (52 years old and 170cm in height) had HT but his thyroid hormones were within the normal range at the several points of time. Her two brothers (18 years old, 166cm and 23 years old, 156cm) were also euthyroid and their anti-thyroid antibodies were negative.
Occasional SITSH

Table 1 The effect of L-T3 on the clinical markers

<table>
<thead>
<tr>
<th></th>
<th>0μg of T3</th>
<th>50μg of T3</th>
<th>100μg of T3</th>
<th>Response at 100μg of T3</th>
<th>Reference response at 100μg of T3 [5]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>89</td>
<td>88</td>
<td>77</td>
<td>87%</td>
<td>&gt;119%</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>51.5</td>
<td>51.3</td>
<td>51.1</td>
<td>99%</td>
<td>&lt;98%</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>250</td>
<td>218</td>
<td>216</td>
<td>86%</td>
<td>&lt;74%</td>
</tr>
<tr>
<td>CK (IU/L)</td>
<td>87</td>
<td>71</td>
<td>67</td>
<td>77%</td>
<td>&lt;68%</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>169.6</td>
<td>145.2</td>
<td>156.2</td>
<td>92%</td>
<td>&gt;120%</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>26</td>
<td>32</td>
<td>42</td>
<td>+16</td>
<td>+9±5</td>
</tr>
<tr>
<td>TSH (mU/L) response to TRH (peak - initial)</td>
<td>10.57-0.73</td>
<td>1.68-0.06</td>
<td>0.54-0.04</td>
<td>+0.5</td>
<td>+5±2</td>
</tr>
</tbody>
</table>

The effects of L-T3 on the clinical markers, such as sleeping heart rate, body weight, total cholesterol, creatine kinase, ferritin, SHBG and TSH response to TRH injection were listed. Responses at 100μg of T3 were compared to the reference responses stated in the literature.

determined by electro-chemiluminescence immunoassay (ECLIA, Roche Diagnostics, Tokyo, Japan). The TgAb and TPOAb were measured with commercial radioimmuno assays (RIA) (Cosmic Corp., Cardiff, UK). The TRAb was assayed with a commercial electro-chemiluminescence immunoassay (ECLIA) (Roche Diagnostics K.K., Tokyo, Japan). The BMD was assessed using the dual energy x-ray absorptiometry (DXA) (Discovery, Hologic Inc., Japan).

**Genetic Studies**

The proband, her brothers and parents provided written informed consent. Genomic DNA was extracted from peripheral blood leukocytes using an EZI DNA Blood 200 μl kit (Qiagen, Hilden, Germany). The specific amplifications for the LBD of TRβ gene (THRB) were performed using the sets of primers as shown elsewhere [3]. Sequencing was performed using a BigDye Terminator Cycle Sequencing kit and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

**T3 suppression test**

After obtained her informed consent, the proband’s mother was administered with liothyronine (L-T3) orally at the dose of 50μg daily from day 1 to day 3 and 100μg daily from day 4 to day 6, according to the previously reported method [9]. 200μg of T3 was not administered because she had hypertension and tachycardia. The TSH-releasing hormone (TRH) injection test was performed with the administration of 0.2mg of protirelin tartrate intravenously and measured TSH and prolactin before and 30, 60, 90 and 120 min after injection at day 1, day 4 and day 7 before administration of L-T3 in the morning. Her body weight (BW) and pulse rate while sleeping (PR) were monitored, and serum CK, TC, ferritin and SHBG were measured at day 1, day 4 and day 7.

The 75-year old female patient with HT who showed SITSH due to anti-T4 antibodies was performed T3 suppression test in another occasion. Her FT4 was 4.3 ng/dL and TSH was 0.35 mU/L. Her data were presented only for the comparison.

The results of TRH test are shown in Fig. 2. The initial and response levels of TSH to TRH were normal without L-T3, compared to the control patient as mentioned above. However, responses of TSH to TRH, after the 3-day administration of 50μg daily or 100μg daily of L-T3, were higher than the control, suggesting that the pituitary resistance to thyroid hormone also exists.

**Discussion**

Here, we described the clinical manifestations of RTHβ patients with heterologous p.R316C mutation.
able even among the same family members, as it is suggested that genetic variability of factors in addition to TRβ gene mutation may affect the phenotype of GRTH [12]. Recently, Ferrara et al. [8] reported a severe RTH patient with homozygous p.R316C mutations. Her relatives with a heterozygous p.R316C mutation showed mild SITSH with a 1.2 fold of upper limit of FT4 level.

To date, over 3,000 affected individuals belonging to approximately 1000 families have been identified [13], harboring mutations in the TRβ gene. Most of the mutations are substitutions of a single amino acid in the LBD of TRβ gene and forms 3 clusters [14]. To compare the degree of SITSH among RTH mutations, we tried to obtain all the data using PubMed search (http://www.ncbi.nlm.nih.gov/pubmed/). As a result, 90 different point mutations with 59 different amino acid positions were obtained (insertions, deletions, and truncations were excluded). About 450 reported values of FT4 and TSH in the literatures were expressed as the percentage to the upper limit of reference range in each institution, and were plotted in Fig. 3A (homozygous cases were not included). There were no apparent differences in the distribution among 3 clusters. The p.R316C including present two cases (expressed as ‘x’) distributed to the area around the upper limit of FT4 and the lower half of TSH. In contrast, the p.R316H (expressed as ‘+’) distributed to the more right area, which represents more severe SITSH. Some mutations were plotted within the normal area of FT4 and TSH (Fig. 3B). For example, p.E311K was reported as a homozygous mutation and the both of heterozygous parents had normal thyroid functions [15]. The p.P247L and the p.R320C, within the normal area of FT4 and TSH, were one of six [16] and one of nine relatives [17], respectively, but all the other members showed apparent SITSH. Two points of p.R429W were from a case who spontaneously improved [18]. The authors speculated that the fluctuant phenotype of the proband suggests that environmental, epigenetic factors may modulate the degree of tissue resistance that is under genetic control and this can be adapted to some particular TRβ mutations exhibit normal thyroid function. However, in our cases, at least epigenetic factors were unlikely because the phenotype did not disappeared but just fluctuated. Although some environmental factors may contribute their fluctuations, our patients and we were unaware of such environmental changes. We speculate that some small occasional variations in their thyroid function, just like usually experi-

The responses to L-T3 of peripheral and pituitary parameters were impaired. However, SITSH, which is a sensitive hallmark of RTHβ, was very mild both in the proband and her mother. Both of them showed occasional SITSH (5 out of 15 occasions of thyroid function test) (Fig. 1A and 1B). The molecular characteristics of p.R316C were compared with a common mutant, p.R316H, using in vitro studies by Nakajima et al. [7]. The binding of p.R316C to T3 was significantly decreased to 38% that of the wild type. The p.R316C did not form a homodimer, but formed a heterodimer with RXR, like p.R316H. Reduced binding of p.R316C with nuclear corepressors (NCoR) in the absence of T3 and impaired release in the presence of T3 were observed. The p.R316C had severe impairment of transcriptional activity on genes both positively and negatively regulated by T3, unlike R316H. It also had a clear dominant negative effect on genes negatively (TRH and TSHβ), but not positively, regulated by T3. This may explain relatively good response of SHBG to T3 in our case. In the case of p.R316C, Adams et al. [10] reported that a family with p.R316H had goiters but were relatively asymptomatic. Three individuals (the proband, sister and mother) had up to 1.5 fold of upper limit of FT4 levels. In contrast, Geffner et al. [11] reported a severe RTH patient with a heterozygous p.R316H mutation. She had a 3.2 fold of upper limit of FT4 level, but her two family members were euthyroid. Thus, the clinical features are var-

![Fig. 2 TRH test with T3 suppression on the proband’s mother](image-url)

The response levels of TSH to TRH were measured before, after 3-day administration of 50μg daily, and additional 3-day administration of 100μg daily of L-T3 on the proband’s mother and the control.
Occasional SITSH

There were no reciprocal relations between increase of thyroid hormones and decrease of TSH. Instead, it seems to be roughly a simple parallel translation. The p.R438H was a case with a pituitary tumor and her FT4 and TSH were within normal range, whereas FT3 was as high as 606 pg/dL (normal range, 210-440) [19].

The p.I54V is probably the first mutation found in the exon 4 of TRβ, which belongs to the amino terminal AF-1 domain of the receptor [20]. However, the thyroid function of the proband was subclinical hypothyroidism (p.I54V in Fig. 3A) rather than SITSH and her family members were euthyroid (p.I54V in Fig. 3B). Because there is no functional analysis with the particular mutant or detailed clinical assessment, it is controversial whether this mutation contributes to RTH or not. Anyway, the patients with a certain RTHβ, espe-

**Fig. 3** Distribution of FT4 and TSH with point mutations in the TRβ collected from the literature

We searched in PubMed using the words «RTH» and «beta». About 220 articles were hit and we selected case reports first. In addition, we also picked up the references that are cited in clinical research papers. The references that used to plot Fig.3 are listed in the Supplemental Table. Ninety different point mutations with 59 different amino acid positions were obtained. The values of homozygous patients or treated patients, such as after thyroidectomy or under some medications affecting thyroid function, were excluded. On the other hand, multiple measurements from one patient were all included. (A) About 450 sets of FT4 and TSH values, expressed as the percentage to the upper limit of reference range in each institution, were plotted. The squares, circles, and triangles indicate clusters #1 (234-283), #2 (310-383) and #3 (429-460), respectively. (B) An enlargement around the normal range of Fig. 3A. The comments on the several mutants within the normal range were described in the test.
from the previously reported cases that showed normal cases had a different phenotype in terms of fluctuation, may also show occasional SITSH if their thyroid function do not exhibit SITSH phenotype as described above, they may also show occasional SITSH if their thyroid function are followed up more frequently. However, our cases had a different phenotype in terms of fluctuation from the previously reported cases that showed normal thyroid function or improved SITSH spontaneously. Therefore, many patients who present occasional SITSH might also remain undiagnosed. Alternatively, only one time SITSH may try to tell us that ‘I am an RTHβ’.

Acknowledgments

We are grateful to Ms. M. Kashiwabara for her technical assistance. We also thank to Drs. Azusa Iguchi and Hanae Hagiwara for their clinical assistance.

Disclosure

All authors have nothing to declare.


Minerva Pediatr 62:419-422.

Occasional SITSH 257


- Safer JD, Colan SD, Fraser LM, Wondisford FE. A pituitary tumor in


References


2. Weiss RE, Hayashi Y, Nagaya T, Petty KJ, Murata Y, et al. (1996) Dominant inheritance of resistance to thyroid hormone not linked to defects in the thyroid hormone receptor alpha or beta genes may be due to a defective cofactor. J Clin Endocrinol Metab 81: 4196-4203.


