Mutation of a Gene for Thyroid Transcription Factor-1 (TITF1) in a Patient with Clinical Features of Resistance to Thyrotropin

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Abstract. Resistance to TSH (RTSH [MIM 275200]) is a heterogeneous condition defined by variable degree of insensitivity to biologically active TSH. While this condition is classically caused by loss-of-function mutations of the TSH receptor gene (TSHR), several patients have exhibited RTSH-like phenotype in the apparent absence of TSHR mutations, and some of them have mutations of PAX8 or GNAS1. We identified a Japanese boy with congenital hypothyroidism who suffered from recurrent lower respiratory infection during infancy and choreoathetosis at a later age. At 14 years of age, he was diagnosed as having RTSH, on the basis of compensated hypothyroidism (TSH, 30.2 mU/L; FT4, 1.2 ng/dl), disproportionate increments of thyroid hormones and TSH during a TRH test (ΔFT3, 0.4 pg/ml; ΔT3, 13 ng/dl; and ΔTSH, 88.3 mU/L), and normal ultrasound thyroid image and radioactive iodine uptakes. Molecular analysis for TITF1 revealed a novel de novo heterozygous deletion/insertion mutation (c.470_479delinsGCG,) that is predicted to lose the entire homeodomain and the NK2-specific domain. We suggest that a heterozygous loss-of-function TITF1 mutation can also cause RTSH-compatible phenotype.

Key words: TITF1, Congenital hypothyroidism, Resistance to TSH

RESISTANCE to TSH (RTSH) is a heterogeneous condition defined by a variable degree of insensitivity to biologically active TSH. While this condition is classically caused by loss-of-function mutations of TSH receptor (TSHR) [1, 2], it also results from alteration in the genes involved in the TSHR-mediated signaling such as G-protein α-subunit [1, 2] and PAX8 [2]. In addition, mutations of thyroid transcription factors such as TITF1 (thyroid transcription factor 1, alias NKX2.1), PAX8, and FOXE1 may also lead to RTSH phenotype, because they are known to reduce the expression of TSHR.

TITF1 is a member of the NK-2 gene family of transcription factors containing a homeobox domain [3]. TITF1 plays a critical role in the development of the embryonic organs including thyroid, basal ganglia, and lung [3], and in the thyroid hormone biosynthesis in postnatal life. For example, TITF1 regulates the expression of thyroglobulin (TG), thyroperoxidase (TPO), and TSHR [4]. As TITF1 regulates TSHR expression, it is possible that RTSH can be caused by TITF1 mutations. Here, we report a patient with RTSH-compatible phenotype and a TITF1 mutation.

Case report

The Japanese patient was delivered by a cesarean section due to breech presentation at 39 weeks of gestation. At birth, his weight was 2960 g (−0.7 SD), and his length 48 cm (−0.8 SD). His non-consanguine-
ous parents and elder brother were clinically normal. Newborn mass screening revealed a borderline TSH level (9.1 mU/L) (upper limit of normal for age, 8 mU/L). While serum TSH and T4 levels were normal (1.2 mU/L and 10.9 μg/dl, respectively) at 1 month of age, serum TSH level was elevated to 41.8 mU/L with normal serum FT4 level (1.7 ng/dl) at 4 months of age; thus, levothyroxine (l-T4) therapy was initiated for compensated hypothyroidism. Although biochemical euthyroidism was maintained by the treatment, he showed a slight delay in psychomotor development and hypotonia. He could not walk without support until 2 years of age, and developed rapid choreoathetosis of trunk and limb which worsened with intentional movements during childhood. Magnetic resonance imaging of the brain revealed no abnormalities, and the neurological symptoms gradually improved by the early teens. In addition, while he had no respiratory problems during the neonatal period, he required hospitalization due to recurrent lower respiratory infections during infancy.

At 14 years of age, he received a comprehensive evaluation. Physical examination revealed a well-nourished boy with a height of 160 cm (–1.0 SD) and a weight of 53.2 kg (–0.3 SD). There was no palpable goiter or features of Albright’s hereditary osteodystrophy. He had subtle choreiform movement in upper limbs and trunk. His walking was wide-based, and tandem gait was very poor. Cranial nerve examination and manual muscle test were normal. He obtained a WISC-III verbal score of 110, performance score of 92, and a full scale score of 101. Furthermore, we performed differential diagnosis for the hypothyroidism. While TSH was suppressed within normal range by oral l-T4 (100 μg/day), discontinuance of the l-T4 supplementation for 4 weeks resulted in an increased serum TSH level (30.2 mU/L) (normal range 0.5–5.0) in association with normal serum levels of FT3 (3.3 pg/ml) and FT4 (1.2 ng/dl). A TRH test showed an exaggerated TSH response (ΔTSH, 88.3 mU/L) and compromised FT3 and T3 responses (ΔT3, 13 ng/dl; ΔFT3, 0.4 pg/ml) (normal range: ΔT3, >25; ΔFT3, >0.6 [5]). Anti-thyroid autoantibodies were all negative. Thyroid ultrasonography was normal in size and position, and 123I scintigraphy showed normal uptake (7.9% at 4 hours, 17.5% at 24 hours). A perchlorate discharge test was not done. Saliva to plasma ratio of radioactive iodine was 24.1, indicating normal iodide transportation. On the basis of the above findings, he was diagnosed as having nongoitrous congenital hypothyroidism due to RTSH. His parents and elder brother had normal thyroid function with negative thyroid autoantibodies and no abnormal neurological signs (not shown).

**Molecular analysis**

This study was approved by the Institutional Review Board Committee at Niigata University School of Medicine. After obtaining written informed consent from the patient and his parents, the coding exons and flanking introns of TITF1 and TSHR were analyzed by direct sequencing, using leukocyte genomic DNA. To confirm a heterozygous mutation, the mutant and the normal alleles were subcloned with a TOPO TA Cloning Kit (Invitrogen), and were subjected to direct sequencing.

This patient had a heterozygous 10 base-pair deletion and 3 base-pair insertion at a position 470_479 in the TITF1 gene (c.470_479delinsGCG, p.P157fsX196) (Fig. 1). This mutation results in a frameshift that is predicted to replace the 215 C-terminal amino acids with 38 additional amino acids. It was absent in 100 control alleles, his parents, and elder brother. No mutation was identified for TSHR (data not shown).

**Discussion**

This patient had RTSH-compatible phenotype and a heterozygous TITF1 mutation. Indeed, the phenotypic features of this patient primarily satisfy the diagnostic
criteria for RTSH [1, 2], although it has not been verified that the TSH of this patient retains normal biological activity. Furthermore, since the mutant protein is predicted to lose the entire DNA-binding homeodomain and the NK2-specific domain, it should have a loss of function effect. In this regard, since TITF1 has a transactivation function for TSHR and for some molecules involved in the signaling pathway downstream of the TSHR such as TG and TPO [4], this would argue that the heterozygous loss-of-function mutations of TITF1 can lead to the development of RTSH. While this patient also had choreoatetosis and recurrent lower respiratory infections, such clinical features are known to be characteristic of TITF1 mutations [6, 7].

Table 1. Thyroid phenotype in patients with TITF1 mutations

<table>
<thead>
<tr>
<th>Case</th>
<th>Mutation</th>
<th>Age</th>
<th>TSH (mU/L)</th>
<th>T4 (µg/dL)</th>
<th>Free T4 (ng/dL)</th>
<th>Thyroid structure</th>
<th>Uptake</th>
<th>Assessment</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Whole gene deletion</td>
<td>19 days</td>
<td>48</td>
<td>13.9</td>
<td>NA</td>
<td>Normal*</td>
<td>Low</td>
<td>Compensated CH</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>Whole gene deletion</td>
<td>8 months</td>
<td>45.6</td>
<td>6.2</td>
<td>NA</td>
<td>Normal*</td>
<td>NA</td>
<td>Compensated CH</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Whole gene deletion</td>
<td>16 years</td>
<td>44</td>
<td>5.9</td>
<td>NA</td>
<td>Hypoplastic</td>
<td>NA</td>
<td>CH</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Y86fsX322</td>
<td>2 years</td>
<td>21</td>
<td>Normal</td>
<td>NA</td>
<td>Normal*</td>
<td>NA</td>
<td>Compensated CH</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>C87X</td>
<td>1.5 years</td>
<td>825</td>
<td>&lt;1</td>
<td>NA</td>
<td>Aplastic*</td>
<td>NA</td>
<td>CH</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>IVS2-2A&gt;T</td>
<td>53 years</td>
<td>Normal</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Normal</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>IVS2-2A&gt;G</td>
<td>19 days</td>
<td>24.5</td>
<td>6.3</td>
<td>NA</td>
<td>Normal*</td>
<td>Normal (R), Low (L)</td>
<td>Compensated CH</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>P157RfxX196</td>
<td>14 years</td>
<td>30.2</td>
<td>NA</td>
<td>1.2</td>
<td>Normal*</td>
<td>Normal</td>
<td>Compensated CH</td>
<td>This study</td>
</tr>
<tr>
<td>9</td>
<td>S169X</td>
<td>13 years</td>
<td>55</td>
<td>3.9</td>
<td>NA</td>
<td>Hypoplastic</td>
<td>NA</td>
<td>CH</td>
<td>10</td>
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<tr>
<td>10</td>
<td>E175X</td>
<td>14 months</td>
<td>elevated</td>
<td>NA</td>
<td>NA</td>
<td>Hypoplastic</td>
<td>NA</td>
<td>CH</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>L194fxX198</td>
<td>3 years</td>
<td>12</td>
<td>11</td>
<td>NA</td>
<td>Normal</td>
<td>NA</td>
<td>Compensated CH</td>
<td>10</td>
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<tr>
<td>12</td>
<td>V205F</td>
<td>15 years</td>
<td>122</td>
<td>2.9</td>
<td>NA</td>
<td>Hypoplastic</td>
<td>NA</td>
<td>CH</td>
<td>10</td>
</tr>
<tr>
<td>13</td>
<td>Q219X</td>
<td>30 years</td>
<td>Normal</td>
<td>Normal</td>
<td>NA</td>
<td>Normal*</td>
<td>NA</td>
<td>Normal</td>
<td>14</td>
</tr>
<tr>
<td>14</td>
<td>P275fxX350</td>
<td>18 days</td>
<td>186.2</td>
<td>6.5</td>
<td>NA</td>
<td>Normal*</td>
<td>Low</td>
<td>CH</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>Q287fsX409</td>
<td>16 months</td>
<td>52</td>
<td>6.8</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Compensated CH</td>
<td>7</td>
</tr>
</tbody>
</table>

CH: congenital hypothyroidism, NA: not available, R: right, and L: left.
* Technetium scintigraphy, † Echography.

To the best of our knowledge, the patients with TITF1 mutations or deletions that described thyroid phenotypes have been identified in 14 sporadic or familial cases [6–15] (Table 1). Although thyroid function studies remain poor, most patients have mild (compensated) hypothyroidism with elevated TSH levels and normal or hypoplastic thyroid glands. Thus, it is possible that TITF1 mutation-positive patients frequently manifest RTSH-like phenotypes.

In summary, we identified for the first time a TITF1 mutation in a patient with clinical features of RTSH. Further studies may clarify the frequency of RTSH in TITF1 mutations and the precise underlying mechanisms.

References

congenital hypothyroidism. 43–47 (In Japanese).


