A Mutation in the Thyroid Hormone Receptor Alpha Gene

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of the receptor results in dissociation of the co-repressor complex and recruitment of coactivator proteins, such as steroid receptor coactivator 1 (SRC-1), which mediate hormone-dependent transcriptional regulation.\(^2\)

Here we describe a child with characteristic clinical features of hypothyroidism (growth retardation, developmental retardation, and chronic constipation) and near-normal circulating thyroid hormone levels. She is heterozygous for a de novo \(\text{THRA}\) mutation, generating a mutant protein that inhibits wild-type receptor function in a dominant negative manner, causing some target tissues to be resistant to the action of thyroid hormone.

**CASE REPORT**

A 6-year-old girl of white European origin, born to unrelated parents, presented with growth retardation. At the age of 18 months, her height had been 79 cm (10th percentile), and the deficit had persisted (Fig. 1A, and Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The child also had decreased subischial leg length with a normal sitting height, indicating that the growth deficit affected the lower segment of her body (Fig. 1B). Tooth eruption had been delayed; she had no teeth at 12 months of age, only eight deciduous teeth at 26 months, and no secondary dentition at the age of 6 years. Her weight for age (23.2 kg) was 1.0 SD above average (Fig. 1 in the Supplementary Appendix), resulting in a borderline-high body-mass index (the weight in kilograms divided by the square of the height in meters) of 23.5. Severe constipation was noted after weaning at 7 months, with infrequent bowel movements (every 3 to 7 days), despite combination laxative therapy with senna and macrogol. Mild hypermobility and ligamentous laxity was present in the ankle and knee. Muscle tone was reduced with normal power but with impairment in gross and fine motor coordination, resulting in a slow, broad-based gait, clumsiness, and difficulty with fine motor skills, including an inability to write or draw. Her affect was placid, with slow, monotonous speech, but with no receptive or expressive deficit. Neuropsychological assessment showed restricted adaptive behavior (Adaptive Behavior Assessment System standard score, 63; 0.7th percentile) and significant impairments in selected cognitive domains, with a standard score for visuoperceptual reasoning of 71 (3rd percentile) on the Wechsler Intelligence Scale for Children, fourth edition, and a standard score for working memory of 77 (4th percentile), despite average verbal comprehension (standard score, 93; 32nd percentile).

**METHODS**

**GENETIC STUDIES**

Our institutional ethics committee approved the study, and the patient’s parents provided written informed consent. We performed high-throughput sequencing of a DNA sample from the patient after whole-exome capture (see the Methods section in the Supplementary Appendix). Bioinformatic analysis of sequence data identified novel variants that were linked to the patient’s phenotype. Sanger sequencing verified variant genotypes in the patient and her family and analyzed other coding exons in \(\text{THRA}\), including in 200 alleles from healthy white persons of the same ethnic background.

**FUNCTIONAL ANALYSES OF E403X MUTANT TR\(\alpha\) PROTEIN**

After generation of the E403X mutant TR\(\alpha\)s by site-directed mutagenesis of wild-type receptor complementary DNA, we performed assays of radiolabeled triiodothyronine binding, transactivation, and dominant negative activity, along with the protein–protein (two-hybrid) interaction assay, as described previously (see the Methods section in the Supplementary Appendix).

**EX VIVO STUDIES OF PERIPHERAL-BLOOD MONONUCLEAR CELLS**

We measured wild-type and E403X mutant TR\(\alpha\)1 and TR\(\beta\) and Krüppel-like factor 9 (KLF9) messenger RNAs (mRNAs) in samples of peripheral-blood mononuclear cells (PBMCs) from the patient and control subjects using a quantitative polymerase-chain-reaction (PCR) assay with specific primers (see the Methods section in the Supplementary Appendix).

**RESULTS**

**CLINICAL AND METABOLIC INVESTIGATION**

Measurements of thyroid hormones in the patient showed low-normal or subnormal levels of total thyroxine and free thyroxine, high-normal or elevated levels of total triiodothyronine and free triiodothyronine, and normal levels of thyroid-stimulating hormone (Table 1, and Fig. 3A in the
Supplementary Appendix), resulting in markedly subnormal ratios of free thyroxine to free triiodothyronine (Fig. 3B in the Supplementary Appendix) and of total thyroxine to total triiodothyronine (Fig. 3C in the Supplementary Appendix), with a very low level of circulating reverse triiodothyronine (Table 1). The level of serum thyroxine-binding globulin was normal (23.5 μg per liter...
The basal metabolic rate was measured by means of indirect calorimetry with the use of a ventilated hood. The reference value is the predicted basal metabolic rate of the patient on the basis of her age, sex, and body composition.

Table 1. Biochemical and Metabolic Measurements in the Patient.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>After Thyroxine Treatment</th>
<th>Reference Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxine (μg/dl)</td>
<td>3.3</td>
<td>10.6</td>
<td>7.4–12.1†</td>
</tr>
<tr>
<td>Total</td>
<td>155</td>
<td>260</td>
<td>130–221†</td>
</tr>
<tr>
<td>Free</td>
<td>0.4</td>
<td>0.7</td>
<td>0.3–0.5‡</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>0.07</td>
<td>0.2</td>
<td>0.21–0.37†</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone (mU/liter)</td>
<td>1.04</td>
<td>&lt;0.03</td>
<td>0.8–6.2‡</td>
</tr>
<tr>
<td>Sex hormone–binding globulin (nmol/liter)</td>
<td>146</td>
<td>131</td>
<td>20–81†</td>
</tr>
<tr>
<td>Insulin-like growth factor 1 (ng/ml)</td>
<td>59</td>
<td>96</td>
<td>67–257†</td>
</tr>
<tr>
<td>Pulse (bpm)§</td>
<td>71</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mm Hg)§</td>
<td>82/51</td>
<td>77/43</td>
<td></td>
</tr>
<tr>
<td>Basal metabolic rate (MJ/day)¶</td>
<td>3.49</td>
<td>4.08</td>
<td>4.06</td>
</tr>
</tbody>
</table>

* To convert the values for total thyroxine to nanomoles per liter or free thyroxine to picomoles per liter, multiply by 12.87. To convert the values for total triiodothyronine to nanomoles per liter, multiply by 0.01536. To convert the values for free triiodothyronine to picomoles per liter, multiply by 15.36. To convert the values for insulin-like growth factor 1 to nanomoles per liter, divide by 7.7.
† Values are from 23 healthy control subjects who were matched with the patient according to age, sex, and body-mass index.
‡ Reference ranges in children from 1 to 5 years of age are from Kapelari et al.³
§ Detailed reference values are shown in Figure 4 in the Supplementary Appendix.
¶ The basal metabolic rate was measured by means of indirect calorimetry with the use of a ventilated hood. The reference value is the predicted basal metabolic rate of the patient on the basis of her age, sex, and body composition.

The child’s heart rate and blood pressure were low, with a resting heart rate of 71 beats per minute (1st percentile) (Fig. 4A in the Supplementary Appendix) and blood pressure of 82/51 mm Hg (systolic, 0.4th percentile; diastolic, 25th percentile) (Fig. 4B in the Supplementary Appendix). The basal metabolic rate (3.49 megajoules [MJ] per day) was below normal, but the level of serum sex hormone–binding globulin, a hepatic marker of thyroid hormone action, was markedly elevated (146 nmol per liter). A normal growth hormone response to provocative testing, with a glucagon test peak of 13.1 μg per liter and a clonidine test peak of 11.7 μg per liter (normal value, >10 μg per liter), was associated with slightly subnormal levels of insulin-like growth factor 1 (IGF-1; 59 ng per milliliter) (Table 1).

After thyroxine treatment (at a dose of 50 μg daily for 9 months), levels of free thyroxine, free triiodothyronine, and IGF-1 rose to normal or supraphysiological levels, with full suppression of thyroid-stimulating hormone and normalization of the basal metabolic rate. However, the level of sex hormone–binding globulin remained high, and the pulse rate and blood pressure remained abnormally low (Table 1, and Fig. 4A and 4B in the Supplementary Appendix), as did the growth rate (Fig. 1B, and Fig. 1 in the Supplementary Appendix) and intestinal transit time (data not shown).

**DE NOVO THRA MUTATION IN PROBAND**

Whole-exome sequencing of a DNA sample from the patient identified many nonsynonymous variants inherited from either unaffected parent but only one heterozygous de novo mutation (c1207 G→T, p.E403X) in THRA, a finding that could explain the child’s phenotype (Table 3 in the Supplementary Appendix). Sanger sequencing identified no other abnormalities in the THRA coding region. The mutation was present in DNA isolated from different cells (PBMCs, buccal epithelial tissue, hair follicle, and colon) obtained from the patient (Fig. 5A in the Supplementary Appendix) but was absent in published normal genomes and exomes (see the Supplementary Appendix) and in 200 control alleles (data not shown). The nucleotide change did not affect other transcripts (e.g., TRα2 and Rev-erba) derived from the THRA locus (Fig. 5B in the Supplementary Appendix).

**FUNCTIONAL CHARACTERIZATION OF E403X MUTANT TRα**

The abnormal receptor did not activate a thyroid hormone–responsive reporter gene (Fig. 2A) and mediated substantial repression of basal promoter activity (Fig. 2A, inset), findings that are consistent with negligible binding of radiolabeled triiodothyronine to E403X TRα (data not shown).
Furthermore, when coexpressed, the E403X receptor strongly inhibited transcriptional activation by wild-type TRα in a dominant negative manner (Fig. 2B). TRα was the predominant receptor subtype in PBMCs from the patient and the control subjects (Fig. 7A in the Supplementary Appendix), and quantitation of receptor transcripts in the patient’s cells indicated that E403X TRα mutant mRNA was expressed with a frequency similar to that of wild-type mRNA, with no evidence of nonsense-mediated decay (Fig. 6A, 6B, and 6C in the Supplementary Appendix). When studied ex vivo, both basal and triiodothyronine-induced expression of KLF9, a known thyroid hormone-responsive target gene, was markedly reduced in PBMCs from the patient, as compared with those from control subjects (Fig. 7B in the Supplementary Appendix). Two-hybrid interaction assays showed...
strong recruitment of corepressors (nuclear receptor corepressor and silencing mediator of retinoic acid and thyroid hormone receptor) by E403X mutant TRα, with failure of their hormone-dependent dissociation. Conversely, E403X TRα showed minimal triiodothyronine-dependent association with coactivator SRC-1 (Fig. 8A and 8B in the Supplementary Appendix).

Structural modeling provided a basis for these observations. The mutation that resulted in E403X prematurely truncated TRα, removing its C-terminal α-helix (H12). Such loss of H12 exposes a hydrophobic cleft on the receptor surface that accommodates corepressor, facilitating its recruitment (Fig. 2C). The H12 deletion also entails loss of amino acids that are critical for hormone binding and coactivator recruitment (Fig. 2D and 2E), with failure of these processes resulting in constitutive binding of corepressor by E403X mutant TRα, accounting for its potent transcription inhibitory (dominant negative) activity.

**DISCUSSION**

Our patient had many clinical features that are typical of hypothyroidism but that paradoxically were associated with borderline low thyroxine levels and high triiodothyronine levels. Patent cranial sutures with wormian bones, delayed dentition, femoral epiphysial dysgenesis, and retarded bone age are classic abnormalities in childhood myxedematous hypothyroidism, and retarded bone age is associated with skeletal abnormalities (delayed tooth eruption, linear growth retardation, reduced ratio of free thyroxine to free triiodothyronine. This abnormal ratio, together with the low serum reverse triiodothyronine levels, may reflect altered metabolism of thyroid hormone in our patient. In this context, we note that hepatic levels of type 1 deiodinase enzyme, which converts thyroxine to triiodothyronine, were markedly raised in TRαPV mice. Alternatively, reduced tissue levels of type 3 deiodinase, which catabolizes triiodothyronine and whose expression is
predominantly regulated by TRα, may also be a contributory factor.19

The incidence of resistance to thyroid hormone is estimated to be approximately 1 in 40,000,20 with de novo THRβ mutations in 27% of cases.21 Since both thyroid receptor proteins are highly homologous, the occurrence of human THRα defects may be similar, albeit possibly limited by impaired fertility and increased mortality, which has been observed in both sexes of dominant negative TRα mutant mice.18,22 The phenotype of mice harboring different TRα mutations is not uniform,22 suggesting that the human disorder may also be variable. Nevertheless, we suggest that the combination of hypothyroid features and near-normal thyroid hormone levels but a subnormal ratio of thyroxine to triiodothyronine and low reverse triiodothyronine levels may be a hallmark of the disorder.

On the basis of the patient's intermittently low levels of thyroxine and the responsiveness to thyroid hormone in murine models, she was treated with thyroxine, and her levels of IGF-1 normalized, although there was little improvement in growth and gastrointestinal function. Higher-dose thyroxine therapy or the use of TRα-selective thyromimetic agents23 may be necessary to avoid hyperthyroidism in TRβ-expressing tissues. However, given the triiodothyronine resistance of a target gene in her PBMCs, hormone-mediated relief of repression may not prove to be possible. We speculate that the use of histone deacetylase inhibitors, such as in the treatment of promyelocytic leukemia mediated by a dominant negative nuclear receptor fusion protein,24 may be an alternative therapeutic strategy.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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