A Novel Mutation in THRA Gene Associated With an Atypical Phenotype of Resistance to Thyroid Hormone

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Context: RTHα is a recently discovered resistance to thyroid hormone (RTH) due to mutation of THRA, the gene encoding TRα1, the thyroid hormone receptor. It has been described in a few patients with growth retardation, short stature, and a low free T₄/free T₃ (FT4/FT3) ratio.

Objective: A 27-year-old patient presenting with dwarfism and a low FT4/FT3 ratio was investigated.

Design: Clinical, biochemical, and radiological data were collected. Whole exome sequencing was performed in the patient and her relatives.

Results: The patient exhibited congenital macrocytic anemia and severe bone malformation with growth retardation, dwarfism, clavicular agenesis, and abnormalities of the fingers, toes, and elbow joints. In adulthood, she presented with active behavior, chronic motor diarrhea, and hypocalcemia. Treatment with T₃ led to heart rate acceleration, worsening of diarrhea, and TSH suppression. Low resting energy expenditure normalized on T₃. rT₃, SHBG, and IGF-1 remained normal. A de novo monoallelic missense mutation in THRA was discovered, the N359Y amino acid substitution (c.1075A>T), which affected both the TRα1 and the non-receptor isoform TRα2. The mutant TRα1 had a decrease in transcriptional activity related to decreased T₃ binding and a dominant-negative effect on the wild-type receptor.

Conclusions: This patient presents a new phenotype including more significant bone abnormalities, lower TSH, and higher FT3 levels, without certainty of all her symptoms with the TRα1N359Y mutation. This case suggests that patients with a low FT4/FT3 ratio should be screened for THRA mutations, even if clinical and biological features differ from previous reported cases of RTHα.

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(TR\textsuperscript{1E403X}) was discovered in 2012 after whole exome sequencing (WES) was done in a 6-year-old girl with growth impairment (4). This initial description prompted the report of several other mutations: TR\textsuperscript{1F397K,406X} (5), TR\textsuperscript{1A382S,838X} (6), TR\textsuperscript{1A263V} (7), TR\textsuperscript{1C392X}, TR\textsuperscript{1P398R}, and again TR\textsuperscript{1E403X} in a different family (8). Finally, a large exome sequencing program aimed at identifying genetic causes of autism identified TR\textsuperscript{1R384C} in one patient (9). This brought the number of mutations to seven, found in a total of 13 patients. These patients are heterozygous, and the disease probably mainly reflects the ability of the mutant receptor to exert a dominant-negative effect on the intact TR\textsuperscript{1}.

All amino acid changes occur in the ligand-binding domain of the receptors, and two underlying mechanisms have been identified: TR\textsuperscript{1} binding domain of the receptors, and two underlying mechanisms have been identified: TR\textsuperscript{1A263V} and TR\textsuperscript{1R384C} (10) have reduced affinity for T\textsubscript{3}, whereas the other mutant receptor lacks the ability to recruit transcription coactivators upon ligand due to an alteration of the C-terminal helix (amino acids 397 and 410).

The clinical features of RTH\textsubscript{a} are quite variable and are often reminiscent of congenital hypothyroidism. Most patients have short stature, impaired bone growth, macrocephaly, and coarse facial features. They also have low basal metabolic rates and various neurodevelopmental defects, which lead to motor dyspraxia, slow speech, and sometimes mental retardation or epilepsy. Constipation and anemia are also frequently observed. Because TH levels usually remain within normal range, RTH\textsubscript{a} patients are not necessarily referred to endocrinologists, and it is likely that the disease frequently remains unrecognized by clinicians. In fact, the latest release of the ExAC database (http://exac.broadinstitute.org) contains 68 THRA mis-sense or frameshift mutations carried by anonymous patients, with most of them predicted to alter TR\textsuperscript{1} function. One helpful serum parameter for diagnosis is the free T\textsubscript{4} (FT4)/free T\textsubscript{3} (FT3) ratio, which in most cases is low when compared with carefully matched controls. This ratio currently seems to be the most reliable parameter for diagnosis. Given the small number of reported cases and the clinical variability, this remains uncertain however.

In this report, we describe a young woman with a TR\textsuperscript{1N1359Y} amino acid change that is located in a different region of the ligand-binding domain. This patient had severe skeletal malformation, growth retardation, and congenital anemia. At the age of 12 years, the patient started to develop parathyroid hyperplasia-associated hypercalcemia and then chronic diarrhea. This phenotype, different from the previously reported case, suggests that the manifestations of RTH\textsubscript{a} are not univocal.

**Case Description**

Part of this patient’s history has been previously reported (11). She was born at 37 weeks gestation. Her parents were healthy and nonconsanguineous. Her mother had been previously treated for Graves’ disease but was cured and was free of antithyroid drugs before pregnancy. The patient had two older healthy siblings. She presented with severe intrauterine growth retardation and subsequent failure to thrive. Her final adult size was 1.29 m (−6 standard deviation) with a weight of 27 kg (body mass index, 16.2 kg/m\textsuperscript{2}) (Supplemental Figure 1). She had muscular hypoplasia and a ventricular septal defect that closed spontaneously. She displayed several bone malformations. These included a small fontanel, clavicular agenesis, humero-radial synostosis, syndactyly of toes 4/5, agenesis of the 12th ribs, and scoliosis (Figure 1). Tooth eruption was normal. Retrospectively, several of these dysmorphic features were suggestive of RTH\textsubscript{a}: macrocephaly, hypertelorism, micrognathia, short and large nose, elongated thorax, short limbs, and ovoid-shaped vertebrae. Bilateral congenital hip dislocation was surgically corrected at the age of 10 years. Menarche started at the age of 14 years. She had hypomastia and breast implants. After normal schooling, she obtained an advanced academic degree. She is currently active and self-sufficient.

From the age of 12 years, the patient’s calcium level was elevated, with the PTH level in the upper range of normal, suggesting hyperparathyroidism. When she was 18 years old, the development of renal colic led to the removal of three hyperplastic parathyroid glands. Laboratory testing before surgery showed hypercalcemia (calcium, 118 mg/L (normal [N], 85–105)), PTH at 68 pg/mL (N, 10–65), phosphorus at 26 mg/L (N, 25–45), calcitriol at 440 ng/dl or 22 mg/kg/d (N, <6), and 25-hydroxyvitamin D at 50 nmol/L (N, >23 nmol/L).

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Materials and Methods

All investigations were performed with the patient’s consent. Sanger sequencing of coding sequences for THRA and other genes implicated in defects of TH activity was performed on leukocyte DNA. WES was performed on the leukocyte DNA of the patient and her parents. Her siblings were tested for new variants discovered in the patient using Sanger sequencing. Expression levels of wild-type and mutant TRα/H9251, TRβ/H9252, and TR-target genes were measured in the patient’s fibroblasts. Functional characterizations of mutant TRα/H9251 and TRβ/H9252 were performed in HepG2 and HEK293 cell lines (for details, see Supplemental Data).

Results

Clinical, biochemical, and radiological investigations

The patient was referred to our clinic at the age of 25 years for recurrence of hypercalcemia ranging from 110 to 115 mg/L with recurrent nephrolithiasis over the previous 2 years. Table 1 summarizes the biochemical, metabolic, and clinical measurements performed on two different occasions. The patient received a preventive cholecalciferol supplementation (100 000 IU every 3 mo) and did not have vitamin D deficiency (25-hydroxyvitamin D, 100 nmol/L). The fibroblast growth factor-23 level was elevated (136 IU/mL [N, 36–96]). The patient had bilateral kidney stones and osteopenia (T-score, −2.2 and −2 in the spine and total hip sites, respectively, according to bone densitometry). Ultrasonography was normal, but abnormal parathyroid uptake on the right inferior part was suspected on the technetium-99m-labeled methoxyisobutyl isonitrile scintigraphy.

Resting energy expenditure (REE) was decreased to 80.5% of the predicted value. Body composition (21.3% fat) was within the normal range. Intestinal transit time (measured by the carmine red test) was reduced to 10 hours, whereas esophagogastroduodenoscopy and colonoscopy with staged biopsies were normal. Other biological investigations did not show any organic abnormalities that would explain the appearance of chronic diarrhea in adulthood. Cerebral magnetic resonance imaging, audiogram, and ophthalmological evaluations were normal. The patient’s intellectual quotient (Wechsler Adult Intelligence Scale) was in the low-average range because of arithmetical difficulties and emotiveness during testing (Supplemental Table 1).

The patient’s FT3 level was high-normal, with a normal FT4 level. Although the TSH concentration was normal at the age of 2 years (1 mIU/L), it decreased when she was 17 (0.5 mIU/L) and remained low in adulthood (Supplemental Figure 2). rT3, thyroglobulin, and serum T4-binding globulin levels were within the normal range (Table 1). Among the peripheral markers of TH activity, only cholesterol and triglycerides were low (Table 1). Prolactin, corticotropin, cortisol, gonadotropin, estradiol, and IGF-1 were all within the normal range. Nonregenerative macrocytic anemia had been observed since the neonatal period. The reticulocyte count was in the lower part of the normal range. Laboratory testing did not find hemolysis, vitamin deficiency, or liver disease. Bone marrow photonic and electron microscopy was typical of type 1 congenital dyserythropoietic anemia (CDA-1) (Supplemental Figure 3).
Identification of germline THRA mutation

The karyotype and comparative genomic hybridization array data were normal. Sanger sequencing of exons did not identify mutations in genes that could explain the hypercalcemia (CASR MEN1 and HRPT2) or congenital dyserythropoiesis (CDAN1, SEC23B, and KLF1). Sequencing was extended to gene-encoding transporters (MCT8, MCT10), a selenoprotein necessary for proper T3 metabolism (SECISBP2), and TRβ1/2 (THRB). Because the patient displayed some traits already reported for RTH/H9251, we also sequenced THRA coding sequences. This identified a monoallelic mutation (c.1075C>G) introducing an amino acid change (N359Y) (Supplemental Figure 4A), which alters both TRβ1 and the other THRA encoded proteins, including TRα2. WES, performed for the patient and her parents, confirmed that the THRA variant was the only de novo functional variant in the patient. None of her siblings carried this mutation, as confirmed by Sanger sequencing. The mutation has not been described in 100 control alleles (data not shown) or in the ExAC database 60 000 exomes (http://exac.broadinstitute.org).

Functional properties of mutant TRα1N359Y

The N359Y amino acid substitution was located at a position that is conserved in vertebrates, in an unstructured part of the ligand-binding domain located between two α-helices (Figure 2A). Its consequences on receptor function were not predictable from in silico analysis. We investigated the ability of TRα1N359Y to provide T3-inducible expression of reporter plasmid constructs with either palindromic or direct repeat 4 (DR4) TH response element. In this transient expression assay, TRα1N359Y showed impaired transactivation activity (Figure 2B) consistent with the observation of decreased binding affinity for T3 (Figure 2C). When coexpressed with wild-type TRα1, TRα1N359Y inhibited transcriptional activity in a dominant-negative manner (Figure 2D), although to a lesser extent than the previously reported TRα1E403X. This dominant-negative activity was also observed on TRβ1-mediated transactivation, but again weaker than the one displayed by TRα1E403X (Figure 2E). The TRα1N359Y transcript was expressed in a proportion similar to the wild-type transcript in the patient’s fibroblasts.

Table 1. Biochemical, Metabolic, and Clinical Measurements in the Patient

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<th>Reference Values</th>
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<td>0.8</td>
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<td>Ferritin, ng/mL</td>
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<td>39</td>
<td>20–180</td>
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<td>Osteocalcin, μg/L</td>
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<td>25</td>
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<td>120/70</td>
<td>130/80</td>
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<td>80</td>
<td>90</td>
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<td>REE, kcal/d</td>
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<td>867</td>
<td>971</td>
<td>Predicted, 1035</td>
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Abbreviations: NA, not available; MCV, mean corpuscular volume; EPO, serum erythropoietin level. Baseline measurements 1 and 2 were performed at ages 26 and 27 years, respectively. The third evaluation was performed after treatment with T3, 25 μg/d for 1 month. After withdrawal from T3, biological and clinical measurements returned to basal levels. Parameter, basal metabolic rate measurement methods, and a table of unit conversion are given in Supplemental Data. The predictive value of REE was calculated on the basis of the patient’s age, sex, and body composition.
cells (Supplemental Figure 4B). The protein level in transfected cells, as measured by Western blot, was similar for TRα1N359Y and TRα1E403X, which ruled out the possibility that the mutation could destabilize the mRNA or protein (Supplemental Figure 5, A and B). In vitro-translated TRα1N359Y and TRα1 were equally able to form heterodimers with RXR on a DR4 element (Supplemental Figure 5C). Therefore, although the position of the amino acid change does not provide immediate explanation for the inability of TRα1N359Y to transactivate, it is, at least in large part, due to its reduced ability to bind T3.

The N359Y amino acid substitution was also present in the nonreceptor proteins encoded by THRA. We therefore addressed the possibility that the mutation could alter the known capacity of TRα2 to inhibit TRα1-mediated T3 response in a transient expression assay. We found a significant but weak reduction in this TRα2N359Y inhibitory activity (Supplemental Figure 6A). Interestingly, TRβ1-mediated transactivation was not antagonized by TRα2N359Y, and a potentiation effect could even be seen (Supplemental Figure 6B).

To have a more direct assessment of the influence of the TRα1N359Y mutation on T3-signaling in the patient, we measured the ability of the patient’s derived fibroblasts to respond to ex vivo T3 stimulation. Although still present, the induction of Klf9 mRNA level, a known TRα1 target gene, was significantly reduced (Figure 2F).

Response to T3 therapy

The patient was treated with liothyronine (25 μg/d) for 1 month. A rising T3 level suppressed the TSH level and significantly reduced the FT4 concentration. The REE

Figure 2. Molecular consequences of the N359Y mutation. A, Amino acid sequence of the C-terminal ligand-binding domain of TRα1. The underlined sequence corresponds to the terminal α-helix required for coactivators recruitment. Changes observed in RTHα patients are indicated (fs, frameshift). The arrow indicates the position from which TRα1 and TRα2 diverge. The three-dimensional structure of the ligand-binding domain of TRα1 (32) shows the position of N359 at the opposite site of helix 12, which interacts with coactivators. T3 is in the central ligand-binding pocket. B, Impaired transactivation ability of TRα1N359Y. HepG2 cells were transfected to express both TRα1 and TRα1 plus TRα1N359Y, and a reporter construct in which the expression of the luciferase encoding sequence is driven by a T3 responsive promoter, containing a multimerized TRE/RXR binding site (TRE-F2 palindromic). Luciferase activity was assayed in the presence of increasing concentrations of T3. C, Impaired T3-binding capacity of TRα1N359Y. T3 binding of TRα1 and TRα1N359Y assayed using saturating amounts of radio-labeled T3 (white bars) and challenged with excess (1 μM) of unlabeled T3 (black bars). D, Dominant-negative property of TRα1N359Y vs TRα1. HEK293 cells were transfected to express a fixed amount of Renilla luciferase, TRα1, and increasing amounts (1:1 to 1:3 DNA ratio) of TRα1N359Y or TRα1E403X. A reporter construct in which Firefly luciferase expression was driven from a DR4-containing promoter was included. The ratio between Firefly and Renilla luciferase was measured 24 hours after stimulation with T3 (10 nM). E, Dominant-negative property of TRα1N359Y vs TRβ1. (Same as in panel D.) F, Quantitative real-time PCR showing Klf9 mRNA expression level (relative to Hprt) and its response to T3. Klf9 is a TR-target gene. The patient fibroblasts are compared with a pool of fibroblasts from control individuals. *, P < .05; **, P < .01; ***, P < .001.
normalized, and the SHBG level increased by 45%. Total cholesterol level, low-density lipoprotein, and high-density lipoprotein remained low. However, the diarrhea worsened, and the heart rate increased. Other parameters did not change significantly (Table 1). The response of the patient to T₃, notably the cardiac and metabolic response, is at odds with previous reports for RTHα patients and is an indication that the mutation only provides limited resistance to T₃.

Discussion

We describe here the case of a 25-year-old patient with a de novo heterozygous mutation of the THRA gene. This mutation is associated with a very particular and profoundly altered phenotype. To date, this case appears to be a unique presentation of RTHα (Supplemental Table 2). Some of the traits observed in the patient are similar to the previous descriptions of the disease and are also found in hypothyroid patients. These can therefore be unambiguously attributed to the reduced transactivation ability of TRα1N359Y. Among these traits are growth retardation, relative macrocephaly, reduced basic metabolic rate, and a reduced FT4/FT3 ratio. However, the patient retains sensitivity to T₃ whereas symptoms thought to be typical of RTHα (dyspraxia, low rT₃ level, mental retardation) are absent. Furthermore, the patient presents with specific features that have not been described in other RTHα patients: numerous osteochondral abnormalities, congenital dyserythropoiesis, and hypercalcemia. We will proceed to address the possible relation between these unexpected features and the THRA mutation.

The relation between TRα1N359Y and the observed dyserythropoiesis is not clear. Macrocytic anemia can be observed in hypothyroidism (12). Although most RTHα patients have normocytic anemia, macrocytic anemia was also observed in two cases and may thus be frequent in RTHα (6). The macrocytic anemia observed in our patient may, however, be particular because of its neonatal occurrence and its CDA-1-like appearance. THRA was originally cloned as it is homologous to the v-erbA avian oncogene, which blocks erythroblast differentiation and induces erythroleukemia (13). THRA knockout mice (14, 15) or mouse models of RTHα (16) are defective for fetal, postnatal, and adult erythropoiesis, resulting in mild anemia. Further investigations could address whether the different anemias observed in patients are equivalent or not. In any case, the THRA mutation is likely to be responsible for the CDA-1-like anemia observed in the present case.

Another remarkable distinctive trait of the patient was the severity of the bone disease, which until now appears to be unique. Although other RTHα patients display some bone malformations (8), these have been much less visible. Impaired ossification, without obvious malformation, is observed in THRA knockout mice (17, 18) and mouse models of RTHα (19). This has been attributed to a defect in cartilage growth and delayed maturation (20). The malformations observed in this patient were reminiscent of cleidocranial dysplasia (21) resulting from an alteration of the Wnt/Runx2 pathway (22, 23). Bone malformations have also been sparsely reported in patients with RTHβ, such as bird-like face, craniosenosis, or rib defects (24), although these may be coincidental (25). Finally, although the link between TH and mineral metabolism has been documented (26), hypercalcemia related to parathyroid gland hyperplasia, observed in the present case but not reported for other RTHα patients, is particularly surprising.

Although we cannot rule out the presence of additional mutations in noncoding regions, WES identified the THRA mutation as the only causative de novo mutation in exon sequences. It is thus tempting to speculate that the peculiar position of the N359Y amino acid substitution could explain the unique phenotype features observed in this patient. In particular, we hypothesized that the presence of the N359Y amino acid substitution in TRα2 may have an influence on the pathology. Our in vitro experiments suggest that in some tissues, like liver, where TRβ1 is predominant, TRα2N359Y could make the cells more sensitive to T₃. This might contribute to lowering the circulating level of cholesterol, maintaining a normal level of IGF-1, and lowering the TSH level. The function of TRα2 is, however, unknown. Due to the evolutionary conservation of the exon encoding the C-terminus of TRα2, it is postulated to have some physiological or developmental function. However, mouse genetics cannot support this hypothesis because the hypersensitivity of mice lacking TRα2 could equally be due to the concomitant overexpression of TRα1 because alternate splicing is eliminated in this mouse model (27). It should also be noted that the A263V substitution, also present in TRα1/TTHRA1N359Y, had no visible effect on its in vitro activity (7). Further analysis therefore will be required to clarify the possible influence of TRα2N359Y on the patient phenotype. Additional investigations could explore possibilities based on the other proteins encoded by THRA, the physiological functions of which also remain unclear, including p43 protein (28, 29), p28 (30), and TRΔα1/TRΔα2 (18, 31). In this complex situation, the outcome of the N359Y substitution present on all these proteins is hardly predictable.

The present case description shows that the phenotype associated with RTHα is not univocal and that there can
be partial resistance to T₃. Only the future discovery of new RTHα cases will help to clarify whether the unusual presentation of the reported case remains unique or whether the diversity of the RTHα presentation is still underestimated. In the present situation, the diagnosis of RTHα is problematic because it is not necessarily associated with the typical features of hypothyroidism. A moderate decrease in the FT4/FT3 ratio appears to be one of the few reliable clues that raises suspicion of a THRA mutation.

Acknowledgments

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