Clinical Phenotype of a New Type of Thyroid Hormone Resistance Caused by a Mutation of the TRα1 Receptor: Consequences of LT4 Treatment

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Context: Recently the first patients with inactivating mutations in T_3 receptor (TR)-α1 have been identified. These patients have low free T_4, low T_4, high T_3, low rT_3, and normal TSH serum levels, in combination with growth retardation, delayed bone development, and constipation.

Objective: The aim of the current study was to report the effects of levothyroxine (LT4) treatment on the clinical phenotype of 2 patients (father and daughter) with a heterozygous inactivating mutation in TRα1.

Setting and Participants: Both patients were treated with LT4 for the last 5 years. To evaluate the effect of LT4 treatment, LT4 was withdrawn for 35 days and subsequently reinitiated. Data were collected from medical records, by reanalysis of serum collected over the last 6 years, and by a detailed clinical evaluation.

Results: Treatment with LT4 resulted in a suppression of serum TSH and normalization of serum free T_4 and rT_3, whereas T_3 levels remained elevated in both patients. In addition, there was a normalization of the dyslipidemia as well as a response in serum IGF-I, SHBG, and creatine kinase in the index patient. All these parameters returned to pretreatment values when LT4 was briefly stopped. LT4 also resulted in an improvement of certain clinical features, such as constipation and nerve conductance. However, cognitive and fine motor skill defects remained.

Conclusion: This study reports the consequences of LT4 treatment over a prolonged period of time in 2 of the first patients with a heterozygous mutation in TRα1. LT4 therapy leads to an improvement of certain but not all features of the clinical phenotype. **(*J Clin Endocrinol Metab* 98: 3029–3038, 2013)**

The importance of thyroid hormone (TH) for normal development is illustrated by the severe consequences of untreated congenital hypothyroidism, which results in growth failure and permanent mental retardation. The production of TH by the thyroid gland is regulated by the hypothalamus-pituitary-thyroid (HPT) axis, in which pituitary TSH stimulates the thyroid to produce TH (1). T_4 represents most TH secreted by the thyroid, whereas the biological activity of TH is largely mediated by the binding of the active hormone T_3 to its nuclear T_3 receptor (TR). TRs function as ligand-dependent transcription factors, which regulate target gene expression by binding to T_3 response elements (TREs) in the promoter region (2, 3).

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Abbreviations: BMD, bone mineral density; BP, blood pressure; CK, creatine kinase; D, deiodinase; FT_4, free T_4; HDL, high-density lipoprotein; HPT, hypothalamus-pituitary-thyroid; IQ, intelligence quotient; LDL, low-density lipoprotein; LT4, levothyroxine; MMSE, minimental state examination; TH, thyroid hormone; TR, T_3 receptor; TRE, T_3 response element; WT, wild type.

Different TR isoforms are generated from the *THRA* and *THRB* genes by alternative splicing and different promoter usage, with TRα1, TRβ1, and TRβ2 as highly homologous T_3 binding isoforms (3). TRα1 and TRβ1 are widely expressed, and their expression is spatiotemporally regulated. TRα1 is preferentially expressed in the brain, bone, and heart, whereas TRβ1 is considered the major isoform in the liver, kidney, and thyroid (3, 4). TRβ2 has a more restricted expression pattern, regulating neurosensory development as well as the HPT axis (5, 6).

Heterozygous mutations in the ligand-binding domain of *THRB*, leading to impaired hormone binding and/or transcriptional activity of the receptor result in resistance to TH. Resistance to TH is a syndrome characterized by elevated serum TH levels and a nonsuppressed TSH and a variable phenotype including goiter, tachycardia, and raised energy expenditure (7, 8).

Ever since its characterization in 1987, investigators have searched for patients with mutations in TRα1, which had not been identified until recently. The phenotype of the first patients with inactivating mutations in TRα1 includes abnormal thyroid function tests [low free T_4 (FT_4), high T_3, but normal TSH levels], growth retardation, delayed bone development, and constipation (9, 10). In the current study, we describe the consequences of treatment with levothyroxine (LT4) for different thyroid-related phenotypes in 2 of these patients.

Patients and Methods

Informed consent was obtained from the index patient and her parents. The index patient is a 12-year-old girl from Greece (10). The patient and her father have a heterozygous single-nucleotide insertion in exon 9 of *THRA*, resulting in a frame shift and alteration of the C-terminal domain of TRα1 (F397fs406X). As previously described, the clinical phenotype associated with this mutation includes growth retardation, delayed bone development, and mildly delayed motor and cognitive development (10). Because of hypothyroid symptoms, LT4 treatment was started at 6 years of age in the index patient. The initial LT4 dose was 1.15 μg/kg/d, corresponding to 25 μg LT4 per day. During follow-up, the LT4 dose was adjusted based on serum FT_4 levels to 37.5–45 μg/d. LT4 therapy resulted in a transient increase in growth (10). Because of evidence of GH deficiency, GH therapy was started at 8.5 years of age. The index patient received a fixed dose of 0.15 mg/kg body weight per week, corresponding initially to 4 mg GH per week. At that age brain magnetic resonance imaging was normal and there was no hearing defect. The father received LT4 treatment from 42 years of age in a daily dose of 50–75 μg. Before LT4 treatment, a TRH test with 200 μg TRH was performed in both patients.

To evaluate the effect of LT4 treatment, LT4 was stopped for 35 days at 11 years of age in the girl and at 47 years of age in the father, and a detailed clinical analysis was performed. Seven months after LT4 therapy was reinitiated, clinical analysis was repeated.

Neurological function was evaluated by the following: a questionnaire on neurological symptoms; assessment of the mental status by the minimental state examination (MMSE); a Raven test for intelligence quotient (IQ); evaluation of the visual-spatial orientation with DTVP-A (Developmental Test of Visual-Perception-Adolescent and Adult) cards; intelligence with the Weschler Abbreviated Scale of Intelligence; and a neurological examination, which included the Phalen maneuver (forced complete flexion of the wrist), grading of muscle strength by the Medical Research Council scale, assessment of tendon reflexes and sensory testing of pinprick, and joint position and vibration in feet and hands. In addition, a neurophysiological profile was assessed, which consisted of the following parameters: 1) motor conduction of median, ulnar, and fibular nerves with measurements of distal motor latency, motor conduction velocity, and amplitude of compound muscle action potential; and 2) sensory conduction of median, ulnar, and sural nerves with measurements of distal sensory latency, sensory conduction velocity, and amplitude of sensory action potential. The neurophysiological examination was performed by using standard methods, using surface electrodes and maintaining the limb's temperature between 32°C and 34°C. Dual-energy X-ray absorptiometry was used to measure bone mineral density (BMD) in the lumbar spine and femoral head.

Thyroid function tests

In the last 6 years, serum samples of both patients had been collected before, during, and after treatment with LT4 and/or GH and stored at −20°C until analysis. In all samples we measured serum TSH, FT_4, T_4, and T_3 levels by the Vitros ECiQ (Ortho-Clinical-Diagnostics, Amersham, United Kingdom); rT_3 was measured by a commercially available RIA (Zentech, Angleur, Belgium), and SHBG was measured with the Immulite 2000XPi (Siemens, Breda, The Netherlands). All other measurements [total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, creatinine kinase, hemoglobin, erythropoietin, GH, IGF-I, and prolactin] were obtained during a regular clinical follow-up.

Functional analysis of TRα1-F397fs406X mutation

To understand the in vivo effects of LT4 treatment, we studied the possible dominant-negative effect of mutant TRα1 on wild-type (WT) TRβ1 in vitro, as well as if this dominant-negative effect could be overcome by high T_3 concentrations. These in vitro studies were performed as previously described (10). In brief, HepG2 cells cultured in 96-well plates were cotransfected with 15 ng TRE-luciferase construct, 15 ng TK-renilla (Promega, Leiden, The Netherlands), and 10 ng WT TRα1, mutant TRα1-F397fs406X, or 5 ng WT TRβ1 (10, 11). As we described previously, mutant TRα1 has a dominant-negative effect over WT TRα1 when transfected in a 1:1 ratio. In the current study, we analyzed the effect of cotransfection of mutant TRα1 on the transcriptional activity of WT TRβ1. Cells cotransfected with WT TRα1 and WT TRβ1 in equal ratios were used as a control. After washing and incubation for 24 hours with 0–1000 nM T_3, luciferase and renilla values were determined by a luminometer (Topcount NXT; Packard Instrument Co, Meriden, Connecticut).

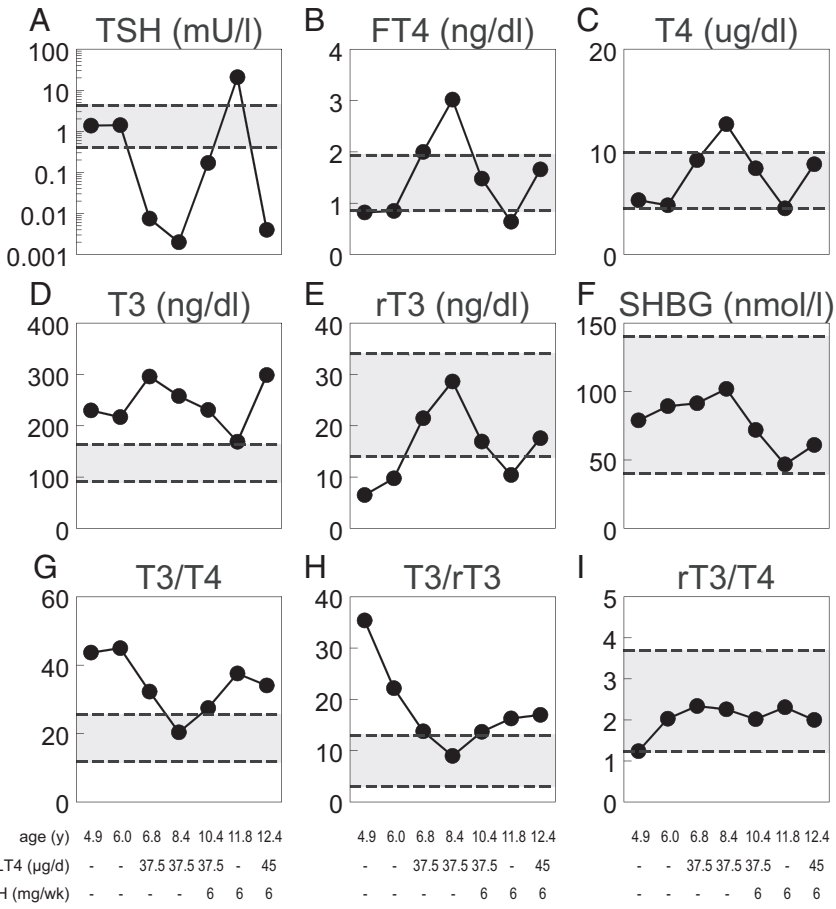

Figure 1. Serum thyroid function tests and SHBG levels in samples that were collected from the index patient under different treatment modalities (on and off LT4 and/or GH therapy) at different time points. A, TSH. B, FT_4. C, T_4. D, T_3. E, rT_3. F, SHBG. G, T_3 to T_4 ratio. H, T_3 to rT_3 ratio. I, rT_3 to T_4 ratio. Horizontal lines represent the different reference ranges.

Results

Serum thyroid function tests and consequences of treatment with LT4

Serum samples had been collected from the index patient and her father during the different periods off and on LT4 and/or GH therapy. In these samples, an extensive thyroid function profile was determined. Results for the index patient are presented in Figure 1. Before treatment, FT_4 and T_4 levels were low-normal, T_3 was increased, and rT_3 was decreased, whereas serum TSH was normal. Treatment with LT4 was started at the age of 6 years. This resulted in a suppression of TSH, an elevation of FT_4 and T_4, and a normalization of rT_3, whereas serum T_3 increased slightly. Additional treatment with GH was started at the age of 8.5 years. This was accompanied by a decrease in FT_4, T_4, T_3, and rT_3 levels, which may also be due to a relative decrease in the dose of LT4 per kilogram body weight. The T_3 to rT_3 ratio and T_3 to T_4 ratio were highly elevated before treatment (Figure 1); they were decreased by LT4 treatment but not affected by GH therapy. The rT_3 to T_4 ratio was in the normal range and did not change during treatment. TSH remained suppressed until LT4 was temporarily stopped at the age of 11 years. This resulted in a steep increase in TSH levels as well as a marked decrease in serum T_4, FT_4, and rT_3 to low-normal levels and T_3 levels to the upper limit of the normal range. Reinitiation of LT4 therapy resulted in a suppression of serum TSH and a normalization of FT_4 and rT_3, whereas T_3 levels increased.

Also, in the father, FT_4 and T_4 levels were low-normal, T_3 was increased, and rT_3 was decreased in combination with a normal TSH before treatment (Figure 2). LT4 treatment resulted in a suppression of serum TSH and a normalization of serum FT_4 and T_4 levels, whereas serum T_3 remained elevated. Serum rT_3 increased but remained in the low-normal range. TSH levels normalized when LT4 was temporarily stopped. LT4 withdrawal resulted in low serum FT_4, T_4, and rT_3 levels, whereas T_3 decreased to the upper limit of the normal range (Figure 2). When LT4 was restarted, TSH and iodothyronine levels returned to their previous treatment values. The elevated T_3 to rT_3 and T_3 to T_4 ratios also decreased by LT4 treatment, whereas the normal rT_3 to T_4 ratio did not change during treatment. Thus, the effects of LT4 therapy on the different iodothyronine levels and their ratios showed a similar pattern in the father and index patient.

The negative feedback of TH on TSH secretion is predominantly regulated by serum FT_4 (12, 13). There was a clear, log-linear negative relationship between TSH and FT_4 in both patients (Figure 3, A and B), suggesting that the negative feedback of FT_4 on TSH secretion is intact. Before LT4 therapy, pituitary function was additionally tested with a TRH test, which showed a subnormal TSH response but normal prolactin response in both patients (Figure 3, C and D).

Serum markers reflecting thyroid state and consequences of treatment with LT4

Total and LDL cholesterol levels, which are increased in hypothyroid patients, were clearly elevated in both patients despite the elevated serum T_3 levels (Figure 4). In the index patient, LT4 treatment resulted in a normalization

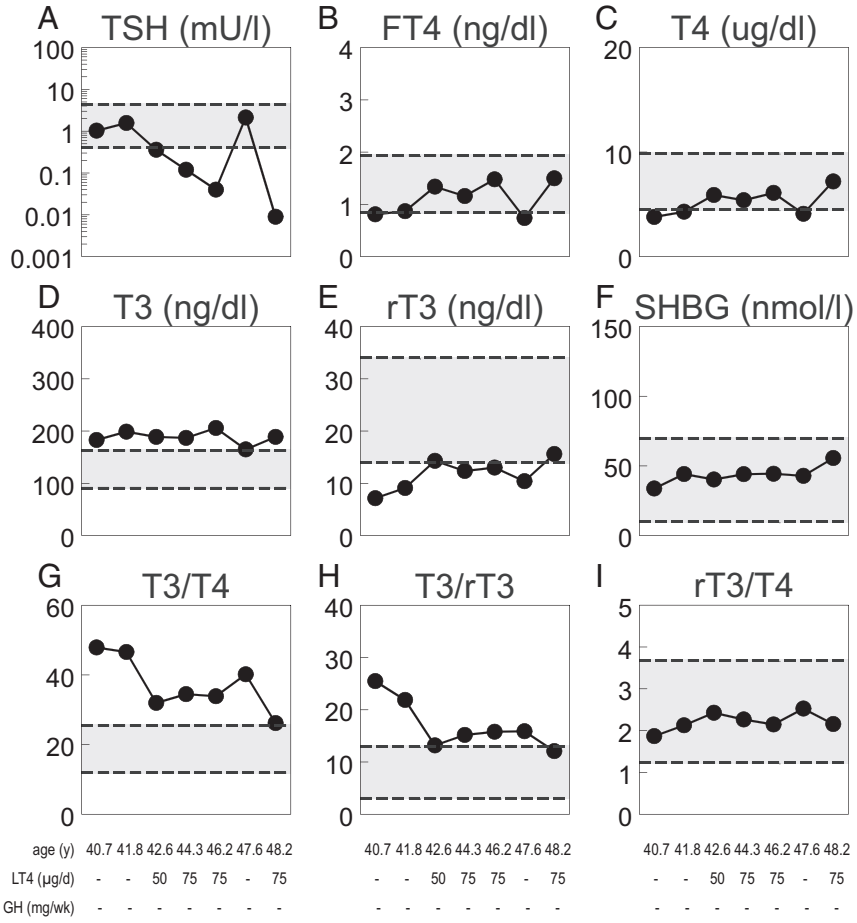

Figure 2. Serum thyroid function tests and SHBG levels in samples that were collected from the father on and off LT4 therapy at different time points. A, TSH. B, FT_4. C, T_4. D, T_3. E, rT_3. F, SHBG. G, T_3 to T_4 ratio. H, T_3 to rT_3 ratio. I, rT_3 to T_4 ratio. Horizontal lines represent the different reference ranges.

of the elevated total and LDL cholesterol levels (Figure 4A). After 35 days of LT4 withdrawal, both total and LDL cholesterol returned to their elevated pretreatment values. Reinitiation of LT4 resulted in a normalization of the serum cholesterol levels. There was a significant negative correlation of total and LDL cholesterol with serum FT_4 levels (Figure 4B) but not with serum T_3 levels (data not shown). The relationship between FT_4 and cholesterol levels in the father could not be studied because he is currently treated with rosuvastatin for his dyslipidemia.

Serum IGF-I levels, which are known to be influenced by thyroid function, were low to low-normal in both patients. IGF-I was increased by LT4 treatment in the index patient but not in the father (Figure 4, C and D). GH stimulation tests were performed during LT4 therapy, showing a subnormal response to both clonidine and levodopa in the index patient and a blunted response to clonidine in the father (data not shown). The IGF-I levels were normalized in the index patient by treatment with LT4 plus GH. Her IGF-I levels decreased in response to LT4 withdrawal and increased again after LT4 continuation (Figure 4C). In the father, no clear changes in IGF-I levels were observed during temporary LT4 withdrawal (Figure 4D).

In the index patient, serum SHBG levels were in the normal range and approximately followed serum FT4 levels in the different treatment periods (Figure 1). Also in the father, serum SHBG was normal, but it showed little response to LT4 treatment (Figure 2). Serum creatine kinase (CK) was clearly responsive to LT4 treatment in the index patient [on-off-on LT4: 149–196-121 U/L (reference range $<$ 190 U/L)], but this was less clear in the father (not shown).

Other laboratory findings were a normocytic anemia with low red blood cell count and low erythropoietin levels in the index patient. Her hemoglobin did not respond to LT4 treatment (11.5 and 10.6 g/dL before and on LT4, respectively). The father had a similar normocytic anemia, which normalized during LT4 treatment (hemoglobin 10 and 14.2 g/dL before and 1 year on LT4, respectively).

Additional description of the clinical phenotype and consequences of LT4 treatment

To study the effect of LT4 treatment on other aspects of the clinical phenotype, both patients underwent a detailed clinical examination 35 days after LT4 withdrawal and 7 months after LT4 treatment was reinitiated. Off LT4, the index patient had a heart rate of 88 ± 3.2 bpm (mean ± SEM, n = 7 measurement on 2 separate days), a normal blood pressure (BP) (106 ± 2.4 over 56 ± 2.6 mm Hg) and normal body temperature (36.2 ± 0.2°C) during admission. Electrocardiography was normal, both in the index patient and her father, with a normal duration of the QRS complex (103 and 110 milliseconds, respectively) as well as a normal corrected QT interval (381 and 414 milliseconds, respectively). Furthermore, echocardiography showed no major clinical abnormalities. At 12 years of age, she was pubertal (breast development Tanner stage II), was pale, and had a small soft goiter and slow deep tendon reflexes. Her height was 137 cm and her weight was 40 kg, corresponding to a high-normal body mass

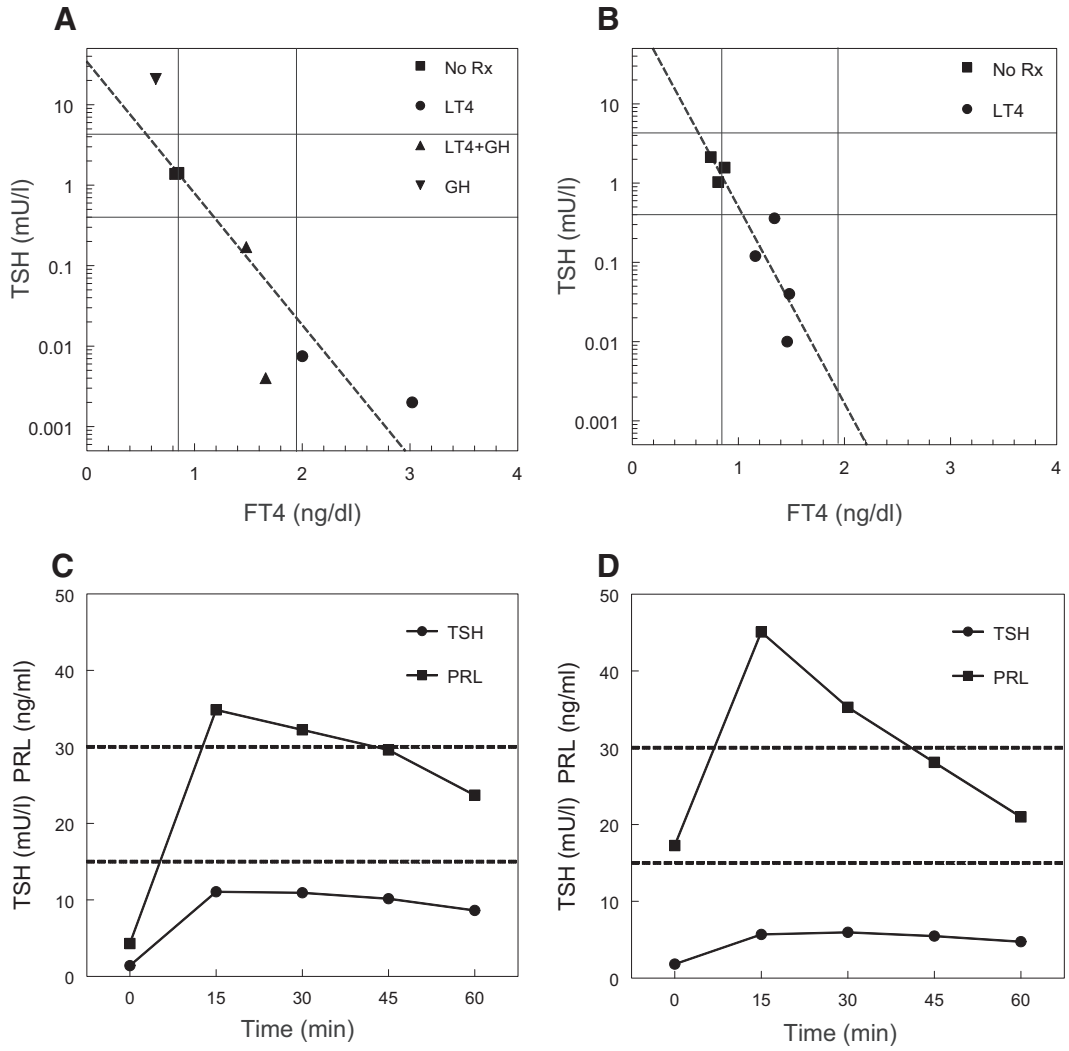

Figure 3. Serum TSH plotted as a function of serum FT_4 levels in the index patient (A) and the father (B). The different symbols represent the different treatment modalities (on and off LT4 and/or GH therapy) of the patients when serum thyroid function tests were determined. The regression line is based on all data points. Reference ranges are indicated by the horizontal and vertical lines. Serum TSH and PRL levels after TRH stimulation in the index patient (C) and father (D) are shown. Rx, medication; PRL, prolactin.

index of 21 kg/m^2. She did not report more drowsiness and did not get tired more rapidly, but as we described previously, she did report more constipation after the LT4 was stopped (stool frequency off LT4 every 2–3 days, whereas it was every day on LT4) (10).

Evaluation by a pediatric neurologist at the age of 11 years when the patient was off LT4 showed that she had mild coarse features and difficulties in running tests requiring coordination. She scored 19 of 30 points on an MMSE test (<23 points indicates mild cognitive impairment). She was delayed in orientation, attention, calculations, and language. Her mental age based on the drawing of a human figure corresponded to a child at the age of 7 years. Her visual-motor coordination corresponded to a girl of 9 years. Her general behavior was characterized by slowness but also by significant improvement of her performance after reinforcement. Memory evaluation by a neuropsychologist revealed that she had mild cognitive deficits, especially to code new materials acoustically and verbally, and mild problems concerning attention and working memory. Her IQ was 90, but her functional ability was normal. Her hearing, evaluated by an ear-nose-throat physician, was normal. In conclusion, she had a 3- to 5-year delay in higher mental functions, corresponding to the age of 7–9 years.

Seven months after the reinitiation of LT4 treatment, she was readmitted for clinical evaluation. Her heart rate had slightly increased to 94 ± 2.0 bpm (n = 7), and the BP was 99 ± 0.6 over 55 ± 1.3 mm Hg. Her body temperature was still 36.2 ± 0.1°C. She reported that the stool frequency had normalized. Breast development had progressed to Tanner stage III. Her neurological and developmental assessment had not improved significantly. She was still behind in orientation, attention, calculations, and language, and she had the same score with the MMSE and IQ test as in the period off LT4. The only difference was

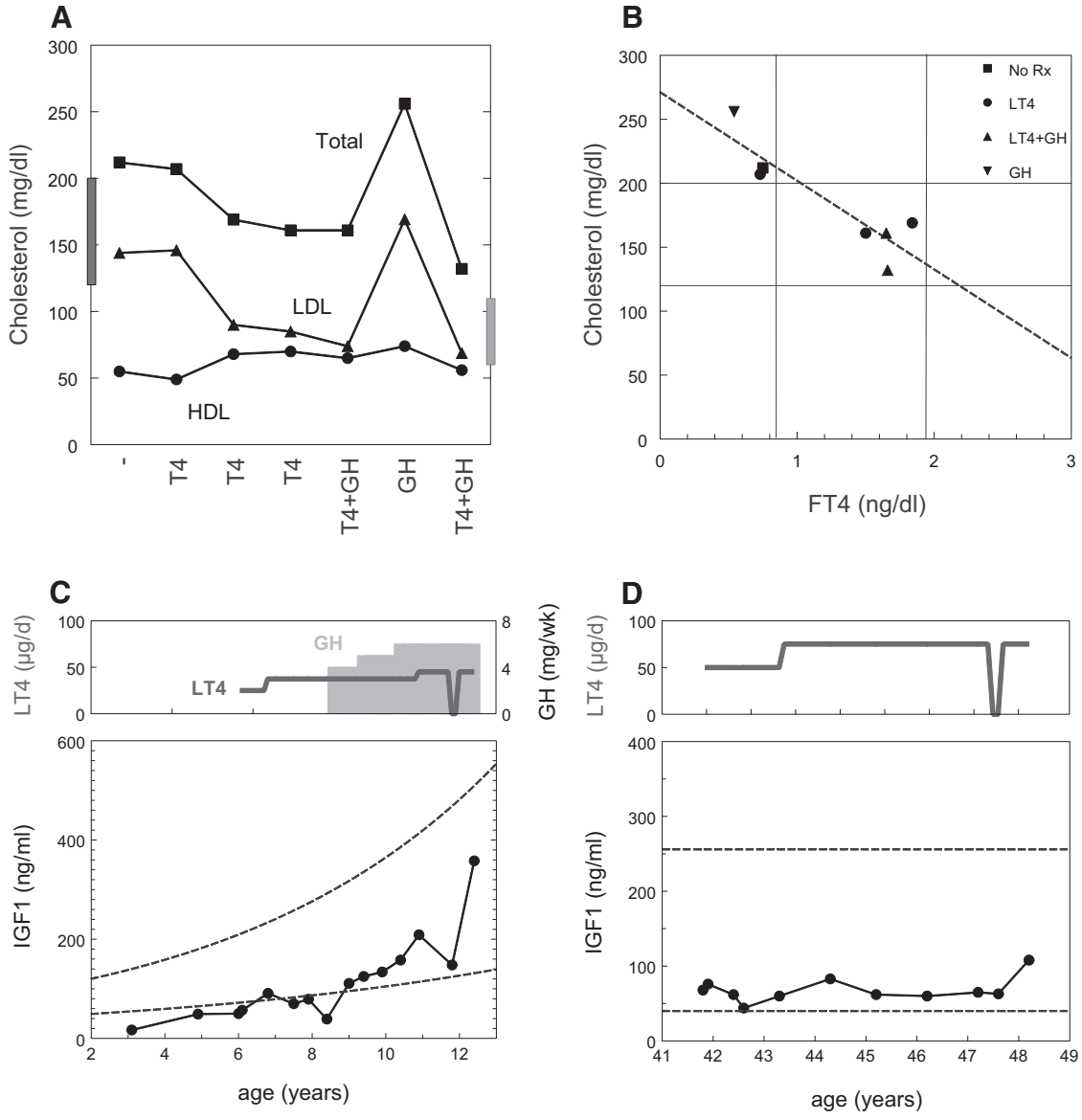

Figure 4. Serum total cholesterol, LDL cholesterol, and HDL cholesterol values in relation to different treatment modalities (on and off LT4 and/or GH therapy) in the index patient. The reference range of total cholesterol is indicated with the shaded area in the left vertical axis, whereas the reference range of LDL cholesterol is indicated with the shaded area in the right vertical axis. The reference range for HDL cholesterol is 30–65 mg/dL (A). Serum total cholesterol was plotted as a function of serum FT_4 during different treatment modalities in the index patient (B). Serum IGF-I levels determined over the years in the index patient (C) and the father (D), in relation to T_4 dose (micrograms per day) and/or GH dose (milligrams per week). Stippled lines indicate IGF-I reference ranges. Rx, medication.

that she was much more energetic and that her defecation pattern had improved.

The father was clearly overweight, with a body mass index of 36 kg/m^2. Also in the father, the heart rate increased after the reinitiation of LT4 treatment (from 75 to 90 bpm), whereas BP (125/70 mm Hg) and body temperature (36.2°C) were unaffected. Neuropsychological evaluation resulted in the same IQ on and off LT4 (IQ = 85). The father has no cognitive deficits, but off LT4 treatment, a mild delay in recall of new materials and processing speed was noted compared with his functioning on LT4 treatment. It should be noted that the father has acquired hearing loss, which is most likely due to otosclerosis (confirmed by computed tomography scan). Because the father used a hearing device during the neurocognitive evaluation, it is unlikely that the hearing deficit influenced the results of the evaluation.

Neurophysiological analysis of the index patient showed mildly affected distal motor latency prolongation and borderline sensory conduction velocity in the median nerve, findings that suggest, in association with the normal measurements in all other nerves, a subclinical carpal tunnel syndrome (Table 1). The patient's father had typical bilateral sensory symptoms of carpal tunnel syndrome, experienced severe difficulty making fine hand movements, and had a positive Phalen maneuver. Clinical ex-

Table 1. Electrophysiological Measurements of the Median Nerve in the Index Patient and Her Father

	Index Patient		Father		
	Off LT4	On LT4	Off LT4	On LT4	Reference Range
Motor conduction study					
Distal motor latency, ms	4.1[a]	3.4	11.8[a]	10[a]	<3.5 (children), <4.1 (adults)
Amplitude of compound muscle action potential, mV	6.2	9.4	2.3[a]	4.5[a]	>5
Motor conduction velocity wrist to elbow, m/s	65	59	51	53	>50
Sensory conduction study					
Distal sensory latency, ms	2.4	2.7	absent[a]	absent[a]	<2.8
Amplitude of sensory action potential, mV	21	15	absent[a]	absent[a]	>7
Sensory conduction velocity wrist-second finger, m/s	51	55	absent[a]	absent[a]	>50

The neurophysiological examination was performed by using standard methods, using surface electrodes, and maintaining the limb's temperature between 32°C and 34°C.

[a] Abnormal data compared to reference range.

amination revealed atrophy of the thenar muscles and slow relaxation of Achilles tendon reflex bilaterally. The findings of the nerve conduction studies supported the diagnosis of a severe bilateral carpal tunnel syndrome in the father (Table 1), whereas measurements in the ulnar nerve were normal in both patients (data not shown). A follow-up neurophysiological examination after LT4 reinitiation showed a normalization of the motor conduction of the median nerve in the index patient as well as a slightly improved (but not normal) motor conduction in the father (Table 1).

In the index patient, BMD was 0.911 g/cm^2 (Z-score +0.3 SD, T-score −0.3 SD) in the lumbar spine and 0.881 g/cm^2 (Z-score +0.3 SD, T-score −1.5 SD) in the femoral head. In addition, the father had a normal BMD of 1.110 g/cm^2 (Z-score +0.5 SD, T-score +0.2 SD) in the lumbar spine and 1.209 g/cm^2 (Z-score +1.5 SD, T-score +1.2 SD) in the femoral head.

Functional analysis of TRα1-F397fs406X mutation

As described previously, the TRα1-F397fs406X mutant showed a complete lack of T_3 activation and a dominant-negative effect toward WT TRα1, which could not be overcome by high concentrations of T_3 (10). However, a clear beneficial effect of LT4 treatment was observed on clinical characteristics such as dyslipidemia and constipation. Because the effects of TH on cholesterol metabolism seem to be mediated predominantly via TRβ (14), we evaluated whether the TRα1-F397fs406X mutant had a dominant-negative effect on TRβ1 as well. Cotransfection studies revealed a dominant-negative effect of mutant TRα1 on WT TRβ1 in the presence of low concentrations of T_3, but in contrast to the dominant-negative effect on WT TRα1, the dominant-negative effect on WT TRβ1 could be partially overcome by higher concentrations T_3 (10–1000 nM) (Figure 5).

Discussion

In the current study, we investigated the consequences of LT4 treatment in 2 of the first patients (father and daughter) with a dominant-negative mutation in the C terminal domain of TRα1 (10). We demonstrate an effect of LT4 treatment on different serum markers reflecting tissue thyroid state (especially total and LDL cholesterol levels) and certain features associated with hypothyroidism but not on cognitive performance or fine motor skills. In vitro analysis showed that mutant TRα1 had a dominant-

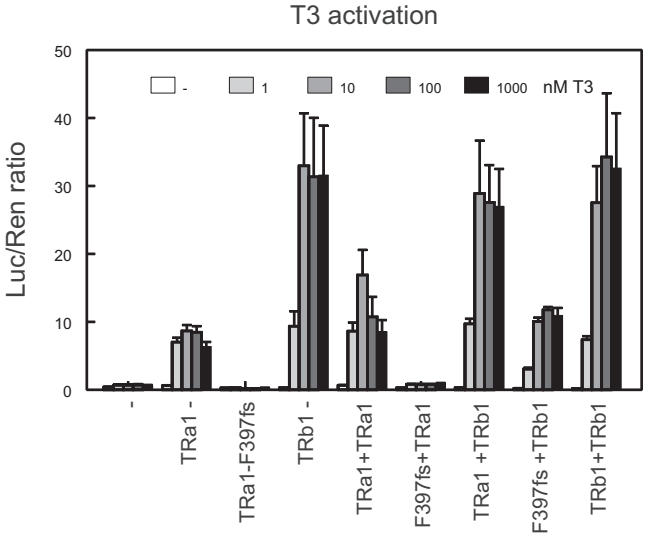

Figure 5. Functional analysis of WT TRα1, WT TRβ1, or TRα1-F397fs406X mutant alone or in combination. The nuclear receptors were cotransfected in cells with a TRE-luciferase reporter construct and TK-renilla, and cells were incubated for 24 hours with increasing concentrations of T_3 (0–1000 nM). As we described previously, mutant TRα1 has a dominant-negative effect over WT TRα1 when transfected in a 1:1 ratio (F397fs+TRa1) (10). In the current study, we analyzed the effect of cotransfection of mutant TRα1 on WT TRβ1 (F397fs+TRb1). Cells cotransfected with double amounts of WT TRα1 (TRa1+TRa1), double amounts of WT TRβ1 (TRb1+TRb1), or a combination of both receptors (TRa1+TRb1) were used as controls. Luc, firefly luciferase activity; Ren, renilla luciferase activity.

negative effect on WT TRβ1, which could be partially overcome by high doses of T_3. For a comparison of the clinical features of patients with inactivating mutations in TRα1 and TRβ, the reader is referred to recent reviews (7–10, 15, 16).

The altered thyroid function tests in all 3 patients with TRα1 mutations that have been described so far is remarkable, considering the primary importance of the TRβ2 isoform in the HPT axis (3, 9, 10). The current study demonstrates that the negative feedback of FT_4 on TSH secretion is intact in both TRα1-F397fs406X patients, which is in line with a predominant role for TRβ2 in the HPT axis (17). In addition, the marked suppression of serum TSH by relatively small increases in serum FT_4 argues for a normal feedback action and thus for a minor role of TRα1 therein. A decreased TSH response was seen after TRH testing despite normal basal levels of TSH. This suggests a decreased sensitivity of the thyrotrophs to TRH, which may be caused by the elevated serum T_3 levels. Despite the normal relationship between TSH and FT_4, both patients have clearly disturbed serum T_4, T_3, and rT_3 levels, and ratios thereof, suggesting altered peripheral TH metabolism by the deiodinases type 1–3 (D1–3) (12, 18–20). As a result of the low FT_4, T_4, and rT_3 levels, the rT_3 to T_4 ratio is in the (low) normal range in both patients.

Interestingly, TRα1PV mutant mice, with a very similar frame-shift mutation in TRα1 as in our patients, have elevated levels of T_3 in combination with a normal TSH as well (21, 22). These mice have markedly increased mRNA and activity levels of liver and kidney D1 (21, 22). Increased activity of D1, which plays a key role in the production of serum T_3 from T_4 and in the degradation of the metabolite rT_3 (18, 23), will contribute to the elevated T_3 to T_4 and T_3 to rT_3 ratios as observed in both patients. Because *D1* is a T_3-responsive gene, the increased D1 expression in these animals may be the cause as well as the consequence of the elevated T_3 levels. However, because D1 activity is not different between TRα1PV mutant and WT mice under hypothyroid conditions, it is more likely that the elevated D1 in TRα1PV mutant mice is the result rather than the cause of the elevated serum T_3 levels (22). In contrast to TRα1PV mutant mice, $TR\alpha1^{-/-}$ mice have normal liver D1 activity (24). Cortex D2 activity, which plays an important role in local T_3 production in the brain, is not different between WT and TRα1PV mutant mice under euthyroid conditions (22).

In addition to an increased D1 activity, a decreased degradation of TH by D3 could also contribute to the high T_3 and low rT_3 levels observed in patients with TRα1 mutations. It has recently been shown that TRα1 mediates the up-regulation of D3 by T_3 and that $TR\alpha1^{-/-}$ mice display an impaired regulation of D3, resulting in a reduced clearance rate of T_4 and in particular T_3 (24, 25). This may contribute to the alterations in serum T_3 and rT_3 levels observed in our patients. Although TRα1PV mutant mice have normal cortex D3 activity under euthyroid conditions, they completely lack T_3-induced D3 expression (22). This results in a decreased T_3 clearance when T_3 levels are high (22). Whether the changes in iodothyronine levels in patients with TRα1 mutations are due to an increased D1 activity, a decreased D3 activity, or combination of both remains to be determined in future studies.

GH treatment was associated with a decrease in T_4 and rT_3 levels. This could be due to the stimulatory effect of GH treatment on D1 activity (26). Nevertheless, the observation that the T_3 to T_4 ratio was not affected by GH treatment argues against this hypothesis. Also, a relative decrease in the dose of LT4 per kilogram of body weight with increasing age may have contributed to the decrease in serum FT_4 and T_3.

Cessation and reinitiation of therapy provided the opportunity to study the direct effects of LT4 treatment on different markers, reflecting tissue thyroid status. Different serum parameters, known to be altered during hyper- and hypothyroidism, were measured. Both patients suffered from clear dyslipidemia, which is remarkable in view of the high T_3 levels. It has been shown in rodents that TRβ is necessary for the stimulatory effects of T_3 on cholesterol metabolism (14), and TRβ selective agonists have lipid-lowering effects in humans (27). However, the dyslipidemia in both patients may suggest an involvement of TRα1 in lipid metabolism as well. Our in vitro data, demonstrating a dominant-negative effect of mutant TRα1 on TRβ1 in transfected cells, suggest that at least part of the dyslipidemia may be caused by dominant-negative effects of mutant TRα1 on hepatic TRβ1 function. Whereas we previously showed that the dominant-negative effect of mutant TRα1 on WT TRα1 was resistant to high levels of T_3 (10), the current study suggests that high doses of T_3 can partially overcome the dominant-negative effects on TRβ1 in vitro. Although it is presently unknown to what extent TRα1 and TRβ1 are expressed in the same human liver cells, this mechanism may very well contribute to the beneficial effects of LT4 treatment on the dyslipidemia in the index patient. However, other causes of the dyslipidemia unrelated to the TRα1 mutation cannot be excluded.

Other serum markers of the thyroid state also showed a response to LT4 therapy. Serum IGF-I levels have been shown to be regulated by TH via direct effects as well as via effects on GH secretion (28). Without a change in the GH treatment, cessation of LT4 therapy resulted in a significant drop in serum IGF-I levels in the index patient. This is in agreement with the findings by Bochukova et al

(9), who reported an increase in the IGF-I levels after LT4 treatment of their patient. In the father, serum IGF-I did not respond to LT4 treatment.

Serum SHBG, of which the hepatic synthesis is stimulated by TH (29), was normal despite the elevated serum T_3 levels. This suggests that the liver may be partially resistant to the high T_3 levels. In contrast, the patient described by Bochukova et al (9) had high SHBG levels, independent of LT4 treatment. On LT4 treatment, CK levels were normal in both of our patients. SHBG levels decreased and CK levels increased in the index patient when LT4 was stopped. The increase in CK levels after LT4 cessation might suggest a relatively low thyroid state in skeletal muscle because serum CK levels increase in hypothyroidism (30, 31). Together, these markers suggest tissue-specific responsiveness to LT4 in patients with TRα1 mutations.

Some clinical parameters also responded to LT4 treatment. The effect on constipation, which clearly improved after LT4 treatment in both patients (10), was most evident and is in agreement with the findings by Bochukova et al (9). In addition, the index patient was more energetic on LT4 therapy, and both patients showed improved motor conductance of the median nerve. The father had severe carpal tunnel syndrome as well, which did not respond to LT4 treatment, possibly because of the chronic nature of the lesion and the secondary degeneration of the nerve fibers. Interestingly, similar findings of carpal tunnel syndrome have also been described in acquired hypothyroidism, predominantly in adults (32). In contrast to the bradycardia reported in the patient of Bochukova et al (9), our patients had a normal heart rate, which appeared to increase on LT4 therapy. However, based on the high serum T_3 levels, a higher heart rate would have been expected (9, 10). This relative bradycardia is in agreement with findings in TR$\alpha^{0/0}$ mice and TRα1-R384C mice (25, 33).

With regard to other features of the clinical phenotype, there was no response to LT4 treatment. In agreement with Bochukova et al (9), neither BP nor body temperature responded to LT4 treatment. TH therapy did not improve the mild cognitive defects of both patients either, nor did it result in a significant improvement of the developmental assessment of the index patient. This lack of effect of LT4 treatment on cognition might be due to the fact that TRα1 is the principal receptor expressed in the brain (34–36) and that the dominant-negative effect of mutant TRα1 on WT TRα1 is not overcome by high levels of T_3 (10). At present, the index patient is still 3–5 years behind with regard to higher mental functions. This may be irreversible, given the importance of TRα1 in early development, because TRα1 is already expressed in brain at week 10 of gestation. Interestingly, TRα1-R384C mice have persistent locomotor deficiencies that can be prevented by early postnatal treatment with TH (37), but TH treatment in adulthood does not improve these locomotor deficiencies. However, in contrast to the TRα1-F397fs406X mutation, which results in a complete lack of T_3 activation, even at very high doses of T_3, the loss of function of TRα1-R384C is overcome by higher T_3 concentrations (37). This suggests that patients with milder loss-of-function mutations in TRα1 may benefit from early LT4 therapy.

Both patients had a normocytic anemia, which is frequently associated with hypothyroidism (38, 39). This is in line with studies in rodents because TR$\alpha^{-/-}$ mice have compromised fetal and adult erythropoiesis (40). Serum levels of erythropoietin were low in both patients, and in the index patient, the anemia did not improve after LT4 therapy, whereas the anemia normalized in the father.

Both patients had a normal BMD compared with age-matched controls. These findings are in contrast to TR$\alpha^{-/-}$ mice, which have osteosclerosis with increased trabecular bone mass at adult age. Moreover, heterozygous mutant TRα1 mice have an even more severe phenotype of increased bone mass (41).

The addition of propylthiouracil to the LT4 therapy of both patients may normalize serum T_3 levels by blocking D1 activity. This could be beneficial by reducing thyrotoxicosis in cells that predominantly express TRβ. However, because we did not see clear clinical features of hyperthyroidism in these patients and because we even observed beneficial effects of LT4 therapy on the dyslipidemia, which is predominantly mediated via TRβ, we have refrained from treating our patients with propylthiouracil.

In conclusion, this report studied the consequences of LT4 treatment in 2 patients with a heterozygous mutation in TRα1, resulting in a new type of reduced sensitivity to TH. Treatment with LT4 led to an improvement of certain features of the phenotype, but cognitive and fine motor skill defects remained. The identification of additional patients will provide more insights into the exact mechanisms involved and also the possible beneficial effects of LT4 treatment.

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