Diverse Genotypes and Phenotypes of Three Novel Thyroid Hormone Receptor-α Mutations

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Context: Recently several patients with resistance to thyroid hormone (RTH)-α due to T_3 receptor-α (TRα) mutations were identified. The phenotype of these patients consists of varying degrees of growth impairment, delayed bone, mental and motor development, constipation, macrocephaly, and near-normal thyroid function tests.

Objective: The objective of the study was to describe the clinical phenotype of three new families with RTHα and thereby gain more detailed knowledge on this novel syndrome.

Design, Setting, and Participants: RTHα was suspected in three index patients from different families. Detailed clinical and biochemical assessment and imaging and genetic analyses were performed in the patients and their relatives. In addition, functional consequences of TRα mutations were investigated in vitro.

Results: We studied 22 individuals from three families and identified 10 patients with heterozygous TRα mutations: C380fs387X, R384H, and A263S, respectively. The frame-shift mutation completely inactivated TRα, whereas the missense mutations produced milder defects. These mutations were associated with decreasing severity of the clinical phenotype: the patient in family 1 showed severe defects in growth, mental, and motor development, whereas the seven patients in family 3 had only mild clinical features. The most frequent abnormalities were anemia, constipation, and a delay in at least one of the developmental milestones. Serum free T_3 ranged from high-normal to high and serum free T_4 and rT_3 from normal to low. TSH levels were normal in all patients.

Conclusions: This large case series underlines the variation in the clinical phenotype of RTHα patients. RTHα should be suspected in subjects when even mild clinical and laboratory features of hypothyroidism are present along with high/high-normal free T_3, low/normal free T_4, and normal TSH. (***J Clin Endocrinol Metab*** **101: 2945–2954, 2016)**

Genetic disorders associated with impaired sensitivity to thyroid hormone (TH) include the following: 1) resistance to TH (RTH)-β due to mutations in *THRB*, which codes for the T_3 receptor (TR)-β1 and TRβ2 isoforms; 2) the Allan-Herndon-Dudley syndrome, a severe neurological disorder caused by mutations in the TH

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Abbreviations: BMI, body mass index; CK, creatinine kinase; FT3, free T_3; FT4, free T_4; RTH, resistance to TH; SDS, SD score; TH, thyroid hormone; TR, T_3 receptor; WT, wild type.

transporter monocarboxylate transporter-8 (*MCT8*); and 3) a multisystem disorder caused by mutations in selenocysteine insertion sequence-binding protein protein 2 (*SBP2*), a protein essential for the production of selenoproteins, including the TH activating and inactivating deiodinases (1–4). Until 2012, no mutation was reported in *THRA* encoding T_3 receptor subtypes, TRα1 and TRα2. Products of the *THRB* and *THRA* genes show differential tissue expression (5–9). TRβ1 is the predominant T_3 receptor in liver, kidney, and thyroid, and TRβ2 is exclusively expressed in hypothalamus, pituitary, retina, and cochlea. On the other hand, growth, development, and function of brain, bone, heart, and intestine are mainly dependent on the interaction of T_3 with TRα1. TRα2 does not bind T_3 and its function is enigmatic.

Since 2012, 15 patients from 10 families have been reported with the RTHα syndrome due to mutations in *THRA* (10–17). The clinical pictures and TH profiles associated with RTHα and RTHβ are consistent with the expression patterns of *THRA* and *THRB*. Elevated serum T_3 and T_4 with nonsuppressed TSH levels are typical for RTHβ. Symptoms are usually mild but may include goiter, tachycardia, atrial fibrillation, mild mental retardation, hyperactivity, and increased resting metabolic rate (1, 2, 4). Patients with RTHα have varying degrees of developmental retardation, growth impairment, and constipation, with low to low-normal free T_4 (FT4), normal to high free T_3 (FT3), and normal to mildly elevated TSH levels (4, 10–15, 17, 18).

Here we present the clinical and laboratory characteristics of the largest case series so far with three novel *THRA* mutations in a total of 10 patients, resulting in a wide spectrum of RTHα phenotypes. More awareness of the variation in the clinical presentation of RTHα is likely to result in the identification of additional RTHα patients.

Patients and Methods

Patients

Three index patients with symptoms and signs suggestive of hypothyroidism associated with near-normal TH levels and their families were included. Detailed information regarding developmental milestones and symptoms of hypothyroidism were collected. The subjects underwent physical examination, including anthropometric measurements, biochemical tests, imaging, genetic studies, and neurodevelopmental tests. The study was approved by the Medical Ethics Committee of Dokuz Eylül University. Written informed consent was obtained from all subjects and/or their parents.

Neurodevelopmental and neuropsychological evaluation

The developmental state was evaluated with the Bayley Scales of Infant and Toddler Development, second edition in children younger than 3 years (19). Older children were tested with Wechsler Intelligence Scale for Children-Revised intelligence tests (20). Adult patients were assessed with Wechsler Adult Intelligence Scale to determine their intelligence quotient score, and classification was done based on the Wechsler classification system (21). A neuropsychological test battery composed of the Mini-Mental State Examination for general cognitive functioning, Oktem Auditory-Verbal Learning Test, Rey-Osterreith Complex Figure Tests for memory and visuospatial skills, and Stroop and Trail Making Tests for executive functioning were applied (22–24). Each test has been shown to be reliable and valid in the Turkish population ,and the details of the procedures can be found elsewhere (25–27). In addition, adult patients were examined for lifetime psychiatric diagnoses and family history.

Laboratory and imaging studies

The thyroid status of all subjects was initially evaluated using measurements of FT4, FT3, and TSH in İzmir (Roche Diagnostics). Total cholesterol, creatinine kinase (CK), complete blood count, ferritin, SHBG, IGF-1, and IGF-binding protein-3 were measured from blood samples obtained between 8:00 AM and 10:00 AM after an overnight fast using routine laboratory methods. Available serum samples were also assayed for TSH, FT4, T_4, T_3 (Vitros ECI Technology, Ortho-Clinical Diagnostics), and rT_3 (RIA; Zentech) levels in Rotterdam. Hip and skull X-rays were obtained.

Identification and functional analysis of THRA mutants

THRA was analyzed for mutations as described (17). FLAG-tagged wild-type (WT) and mutant TRα1 and TRα2 cDNA clones in pcDNA3 were obtained as described (17). Transcriptional activity of WT and mutant TRα1 was assessed using a reporter construct expressing firefly luciferase under the control of a T_3-responsive promoter and renilla luciferase under the control of a constitutive promoter as described (17). The expression of the WT and mutant TRα1 and TRα2 was determined by immunoblotting using an anti-FLAG antibody as described (17).

Statistical tests

Statistical differences between WT and mutant receptors were calculated using a Student's *t* test. Values of $P < .05$ were considered statistically significant.

Results

Clinical assessment

Among three families, 10 patients were identified with *THRA* mutations: six children and four adults, four males and six females, age range from 8 months to 56 years. The pedigrees, photographs, and mutations of the patients are shown in Figure 1. Table 1 presents an overview of the clinical data, and Table 2 (28) summarizes the laboratory data. Results of neurological and neuropsychological tests are provided in Supplemental Table 1. Skull X-ray photographs are presented in Supplemental Figure 1. The fam-

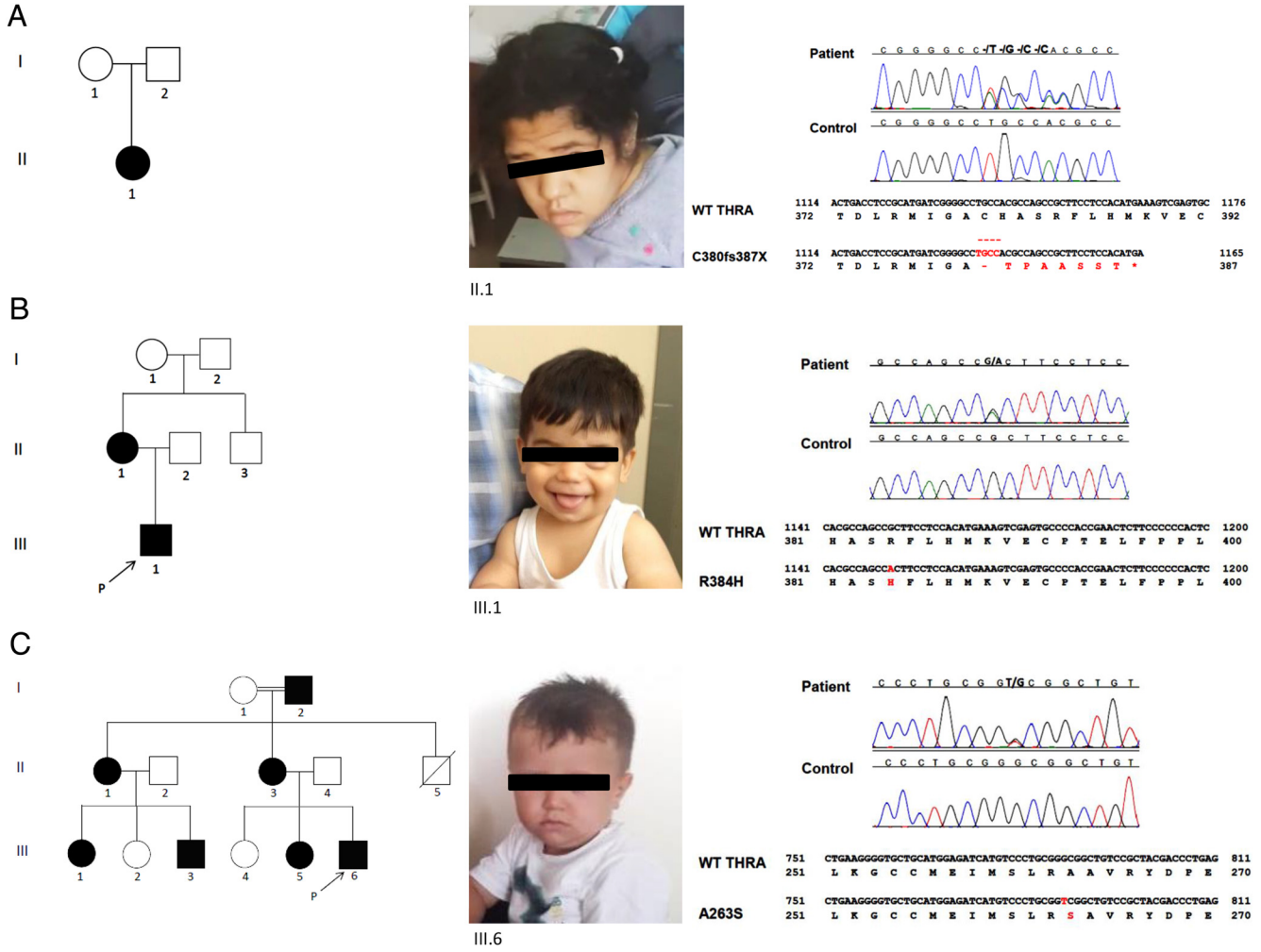

Figure 1. Pedigree and molecular analysis of THRA in the three index patients. A, Sequence analysis of exon 9 of *THRA* shows a deletion of four nucleotides (c.1138–1141delTGCC), resulting in a deletion and frame-shift at codon 380 and an early stop at codon 387 (p.C380fs387X). This mutation is present only in the index patient (1.II.1). B, In both the index patient (2.III.1) and his mother (2.II.1), a nucleotide substitution (c.1151G>A) leads to an arginine to histidine substitution at codon 384 in TRα1 (p.R384H). C, The index patient (3.III.6) and six other members of this family (3.III.1, 3.III.3, 3.III.5, 3.II.1, 3.II.3, 3.I.2) were heterozygous for a nucleotide substitution in *THRA* (c.G787T), leading to an alanine to serine substitution at codon 263 (p.A263S).

ilies are presented in chronological order of diagnosis and evaluation of cases.

Family 1

Patient 1.II.1

The index patient was born at term (birth weight 3200 g) after an eventless pregnancy. First evaluation was made at the age of 16 months due to prominent developmental delay: she could not control her head until the age of 8 months (normal milestone <2 mo [29]); at 16 months she was not able to sit (normal milestone <8 mo [29]) and had no teeth eruption (normal milestone before 13 mo ([30]). In addition, she suffered from constipation and she was noted to have a hoarse-sounding cry, coarse facies, macroglossia, and an umbilical hernia. Normocytic anemia, normal TSH, low FT4, high T_3, and a normal TSH response to TRH (31.8 mU/L at 20 min) were found. At the age of 2 years, she was started on LT4 (4.5 μg/kg · d), and she has been treated since that time with various LT4 doses (1–3.7 μg/kg · d), with a normalization of FT4 levels and suppression of TSH for most of the time. Despite treatment with LT4, she still had a delay in reaching several developmental milestones: sitting occurred at 26 months (normal milestone <8 mo), resolution of umbilical hernia at 3 years, and closure of the anterior fontanelle at 5 years (normal milestone <24 mo [31]). Menarche was at 12 years of age (normal range 12.2 ± 0.9 y [32]). Hypertrophic obstructive cardiomyopathy, pericardial effusion, and nephrolithiasis developed during follow-up.

At her most recent visit at the age of 12.7 years, she was severely handicapped, was not able to walk, and had mild scoliosis. Her height was 127 cm (SD score [SDS] −3.49), weight 43.3 kg (SDS 0.04), body mass index (BMI) 26.85 (SDS 1.81), and head circumference 57.4 cm (SDS 3.12).

Table 1. Clinical Characteristics of Three Families With *THRA* Mutations

	Age, y	M/F	Signs of Hypothyroidism	Height SDS	BMI or WFH SDS	HCF SDS	Sitting/ Total Height SDS	Heart Rate	Blood Pressure	X-Ray Features
Family 1 (C380fs)										
1.II.1 (MT) index	1.3	F	MG, CP, DS, DE, DW, DT, DF, CF, HC	**−2.46**	112% (1.42)	**2.29**	**5.60**[a]	80	120/70[a]	WB, TS, FP
1.I.1 (WT)	33	F	N	−0.98	29.4 (1.49)	0.33	−0.07	84	100/60	N/A
1.I.2 (WT)	37	M	N	−1.65	**32.4 (2.02)**	N/A	N/A	N/A	N/A	N/A
Family 2 (R384H)										
2.III.1 (MT) index	0.9	M	MG, CF (DW, DT, DF)[b]	−0.63	106% (1.25)	**2.02**	−1.00	106	75/40	WB, TS
2.II.1 (MT)	35	F	CP, DE, DW, DT	−1.10	**30.7 (1.60)**	1.33	**3.60**	76	100/70	TS, FP
2.II.3 (WT)	32	M	N	−0.54	26.4 (0.99)	0.00	−0.39	68	120/80	N/A
2.II.2 (WT)	35	M	DE, DW, DT	−1.25	**31.3 (1.84)**	**3.20**	1.20	88	105/65	N[c]
2.I.2 (WT)	70	M	N	−0.96	**38.1 (2.52)**	**2.30**	1.00	70	120/75	TS[c]
2.I.1 (WT)	71	F	N	**−2.33**	**32.8 (1.77)**	−1.33	1.80	82	125/85	N[c]
Family 3 (A263S)										
3.III.6 (MT) index	2.6	M	CP, DE, DF	−1.18	15.9 (−0.33)	1.14	**2.71**	102	80/50	WB, TS
3.III.5 (MT)	7.4	F	N	−1.66	18.4 (1.22)	−0.11	0.29	78	100/65	WB, TS
3.III.3 (MT)	8.8	M	CP, DE, DW, DT	−1.44	17.2 (0.58)	−0.58	0.08	84	95/60	WB, TS
3.III.1 (MT)	17	F	N	−1.21	25.4 (1.11)	−0.60	0.52	84	115/75	FP[c]
3.II.3 (MT)	31	F	CP	−1.71	21.7 (0.02)	**2.00**	−0.77	68	100/60	WB, TS, FP
3.II.1 (MT)	35	F	DW, CP	−1.56	29.9 (1.52)	1.33	−0.40	86	110/70	WB, TS[c]
3.I.2 (MT)	55	M	DS, DW, DF, ST	**−2.70**	**34.3 (2.19)**	**4.00**	1.00	74	125/75	WB, TS, FP, ED
3.III.4 (WT)	10.1	F	N	−0.98	17.5 (0.27)	0.09	−1.70	76	105/60	WB
3.III.2 (WT)	11	F	N	−0.06	19.0 (0.51)	−1.20	0.55	92	115/70	N/A
3.II.4 (WT)	32	M	N	−0.41	28.6 (1.43)	0.00	−0.08	76	110/70	TS, FP
3.II.2 (WT)	40	M	N	−1.51	26.0 (0.99)	0.80	−0.42	N/A	N/A	N/A
3.I.1 (WT)	56	F	N	**−2.90**	**43.6 (2.29)**	**2.00**	−1.60	68	110/65	N[c]

Abbreviations: CF, coarse facies; CP, constipation; DE, delayed tooth eruption; DF, delayed closure of anterior fontanelle; DS, delayed sitting; DT, delayed talking; DW, delayed walking; ED, epiphyseal dysgenesis; F, female; FP, frontal prominence; HC, hoarse cry; HCF, head circumference; M, male; MG, macroglossia; MT, mutant; N, normal; N/A, not available; ST, skin tag; TS, thickened skull; WB, wormian bones; WFH, weight for height. Abnormal values are indicated in bold.

[a] Measured at the age of 12.7 years.

[b] Hip x-ray could not be performed but there was no gait problem.

[c] Observations were made during follow-up at older age.

Serum TSH was suppressed but, despite LT4 treatment, serum T_4 and FT4 levels were at the lower limit of normal, serum rT_3 was decreased, T_3 and FT3 levels were increased, and the T_3 to T_4 and T_3 to rT3 ratios were markedly elevated. General thickening of the skull vault was demonstrated on cranial X-ray, whereas femoral epiphyses were normal.

The mother (1.I.1) and father (1.I.2) of the index patient were not affected.

Family 2

Patient 2.III.1 (index case)

This boy was born after an eventless pregnancy with a birth weight of 3400 g. Macroglossia was evident at birth and was reported to ameliorate with time. He was first evaluated at the age of 8 months due to frequent upper respiratory tract infections. High FT3, low FT4, and a normocytic normochromic anemia were detected. A TRH test revealed a delayed TSH peak (24.3 mU/L) at 60 minutes. Physical examination revealed an anterior fontanelle of 5 × 3 cm (>90th percentile [2.5 cm]) (31), a decreased height (−0.63 SDS), and an increased BMI (1.25 SDS) and head circumference (2.02 SDS). However, umbilical hernia and constipation were absent. Bone age corresponded to 6 months at the chronological age of 8 months. Lateral skull radiograph showed three to four wormian bones at the conjunction of lambdoid and sagittal sutures and thickening of the skull at the frontal and occipital region.

At 16 months of age, the boy was tested for neuromotor and neuropsychological development. His cognitive ability was only moderately impaired, but his motor development was more severely impaired.

Patient 2.II.1

The 35-year-old mother of the index patient also showed delay in reaching several developmental milestones (Table 1). She had her first menstruation at the age of 13 years, and she became pregnant naturally despite a history of irregular menses requiring treatment. In adulthood, a number of characteristics associated with hypothyroidism were present, such as constipation

Table 2. Biochemical Analyses in Three Families With *THRA* Mutations

Subject	TSH, mU/L	FT4, pmol/L	T_4, nmol/L	FT3, pmol/L	T_3, nmol/L	rT_3, nmol/L	T_3 to T_4 Ratio × 100	T_3 to rT_3 Ratio	Hb (Δ), g/dL	CK, U/L	SHBG, nmol/L[a]	IGF-1, ng/mL[b]	Cholesterol, mg/dL<200
n	0.4–4.3	11–25	58–128	3.8–7.6	1.4–2.5	0.22–0.52	1.4–3.1	3.1–13	[c]	30–168			
Family 1 (c380fs387X)													
1.II.1 index (F, 1.3 y, MT)[d]	1.4	**5.1**	**53**	**12.4**	**2.76**	N/A	N/A	N/A	**8.9 (−1.1)**	N/A	N/A	N/A	168
1.I.1 (F, 33 y, WT)[e]	1.17	12.0	**137**	N/A	**2.56**	0.39	1.87	6.6	12 (0.0)	59	**>180**	224	164
1.I.2 (M, 37 y, WT)	2.68	20.6	107	N/A	**2.60**	0.49	2.43	5.3	N/A	N/A	N/A	N/A	N/A
Family 2 (R384H)													
2.III.1 index (M, 0.9 y, MT)	1.89	13.9	107	**8.08**	**5.20**	0.31	**4.86**	**16.8**	**8.6 (−2.4)**	**268**	125	*33.7*	199
2.II.1 (F, 35 y, MT)	2.51	13.6	80	6.30	**3.32**	**0.20**	**4.15**	**16.6**	**11.2 (−0.8)**	125	70.0	*85.3*	167
2.II.3 (M, 32 y, WT)	2.34	13.4	86	5.16	**2.32**	0.26	2.70	8.9	15.7 (2.7)	**193**	N/A	N/A	179
2.II.2 (M, 35 y, WT)	2.51	11.5	65	5.05	2.41	0.24	**3.71**	10.0	14.1 (1.1)	139	36.2	*53.8*	167
2.I.2 (M, 70 y, WT)	0.78	22.0	118	4.01	2.49	**0.55**	2.11	4.5	N/A	153	38.6	N/A	**226**
2.I.1 (F, 71 y, WT)	1.99	18.6	83	4.41	1.58	0.51	1.90	3.1	13.8 (1.8)	39	20.4	N/A	157
Family 3 (A263S)													
3.III.6 index (M, 2.6 y, MT)	2.10	16.4	85	7.28	**3.65**	0.31	**4.29**	11.8	11.6 (0.1)	**236**	127	*31.0*	134
3.III.5 (F, 7.4 y, MT)	1.40	17.6	98	**7.96**	**3.46**	0.27	**3.53**	12.8	**10.8 (−0.7)**	**218**	93.0	78.1	154
3.III.3 (M, 8.8 y, MT)	2.59	16.1	112	6.65	**2.96**	0.24	2.64	12.3	11.8 (0.3)	**240**	123	155	151
3.III.1 (F, 17 y, MT)	2.03	14.4	89	6.65	**2.53**	**0.19**	2.84	**13.3**	**10.9 (−1.1)**	115	38	<u>450</u>	158
3.II.3 (F, 31 y, MT)	0.95	16.1	87	5.94	**2.51**	0.27	2.89	9.3	**9.6 (−2.4)**	87	90.1	172	149
3.II.1 (F, 35 y, MT)	2.44	15.6	**131**	6.16	**3.21**	0.28	2.45	11.5	**10.5 (−1.5)**	125	78.8	168	174
3.I.2 (M, 55 y, MT)	1.58	13.6	98	5.96	**2.57**	0.28	2.64	9.2	13.5 (0.5)	125	24.0	114	**215**
3.III.4 (F, 10.1 y, WT)	1.44	19.5	108	6.50	**2.81**	0.34	2.60	8.3	12.0 (0.5)	115	65.7	165	172
3.III.2 (F, 11 y, WT)	2.19	16.0	114	6.80	**3.14**	0.35	2.75	9.0	13.3 (1.8)	135	110	204	**208**
3.II.4 (M, 32 y, WT)	1.30	19.0	100	5.30	2.05	0.47	2.05	4.4	15.9 (2.9)	119	N/A	N/A	145
3.II.2 (M, 40 y, WT)	1.87	14.4	68	5.90	1.99	0.22	2.91	9.0	14.8 (1.8)	**408**	23.6	174	182
3.I.1 (F, 56 y, WT)[f]	<0.015	**33.9**	**207**	6.57	**2.61**	**0.54**	**1.26**	4.8	13.3 (1.3)	72	71.0	*56*	**255**

Abbreviations: F, female; Hb, hemoglobin; M, male; MT, mutant; N/A, not available. Abnormal values are indicated in bold.

[a] Hemoglobin reference values, lower limit, 0.5–2 years, 11 g/dL; 2–12 years, 11.5 g/dL; older than 12 years, female, 12 g/dL; male, 13 g/dL; Hb levels relative to the lower normal limits are given in parentheses.

[b] SHBG reference values (given in parentheses): adult, nonpregnant females, 18–144 nmol/L; adult males, 10–57 nmol/L; prepubertal males, 31–167 mmol/L; prepubertal females, 43–197 mmol/L.

[c] IGF-1 reference values (given in parentheses) (see reference 29): low values are indicated in italics, and high values are underlined.

[d] Measured during pregnancy.

[e] Measured at the age of 16 months at presentation.

[f] Measured on LT4 therapy for hypothyroidism.

and slow speech. In addition, she showed several features of RTHα, borderline intellectual functioning, impaired executive functioning, and mild deficits in memory. No psychiatric pathology according to the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition, was evident, and the patient did not have a history of a psychiatric disorder. Her parents (2.I.1 and 2.I.2) and only sibling (2.II.2) were not affected.

Comparison of the two patients with the unaffected members indicates that elevated serum T_3 and T_3 to rT3 ratio and anemia are distinctive features of RTHα in this family. Short stature is not a distinctive characteristic, although the affected mother (2.II.1) shows marked disproportionate growth. Head circumference and thickening of the skull are increased and serum IGF-1 is decreased in both patients, but these abnormalities are also observed in the unaffected father (2.II.2) and grandfather (2.I.2) of the index patient.

Family 3

Patient 3.III.6 (index case)

The index case was born at term with a birth weight of 3000 g. He was evaluated first at the age of 13 months due to frequent infections, and low FT4, normal FT3, and TSH levels were detected. He frequently suffered from constipation and had a delay in tooth eruption (normal milestone <13 mo [30] and his anterior fontanelle had closed at 2 y 4 mo (normal milestone <24 mo [31]). Macroglossia, umbilical hernia, and anemia were not present. Physical examination at the age of 2 years 7 months showed mild growth retardation with a disproportionate body habitus and a mildly increased head circumference. Skull radiographs showed thickening of the skull at the occipital region. Neurological and neuropsychological examination at the age of 2 years 8 months yielded scores for cognition and motor development in the normal range.

The index patient has two older sisters, one of whom is also affected. The affected sister (3.III.5) showed relatively mild growth retardation but otherwise normal development. Her serum T_3 levels were higher and hemoglobin was lower than in her unaffected sister. Also, her global intelligence quotient and verbal and performance scores were lower than those of her older unaffected sister (3.III.4), but they were all in the normal range.

The index patient has three cousins, two of whom are also affected. One cousin (3.III.3) had clearly more clinical

features of RTHα than his oldest sister (3.III.1), including delayed developmental milestones and constipation. Like the index patient, he showed a decreased serum IGF-1 level. He also revealed low average global, verbal, and performance intelligence quotient scores. Both affected cousins had anemia.

The two mothers of the above-mentioned children (3.II.1, 3.II.3) were affected. Both patients suffered from constipation and had anemia. In addition, radiological evaluation of the patients' skull X-rays showed a thickening of the skull vault in combination with wormian bones. The mother of the index patient (3.II.3) demonstrated low-average intellectual functioning, with a more prominent decrease in performance than in verbal intelligence quotient. The patient reported a period of antidepressant therapy after the suicide of her younger brother, but no psychiatric pathology was evident at the time of the interview. We were not able to evaluate any biological samples from the deceased brother (3.II.5), but his psychiatric story was insignificant before he committed suicide. Patient 3.II.1 had also received treatment for depression and panic attacks.

The index patient's grandfather (3.1.2) was also affected and showed several RTHα characteristics during development as well as short stature and marked macrocephaly. He displayed low-average intellectual and verbal memory and executive functioning. The patient reported a depressive episode, during which he used antidepressant medication for a year, after the suicide of his son (3.II.5).

The grandparents are consanguineous. The grandmother (3.I.1) is not affected, although she has long-standing hypothyroidism from an unknown cause for which she receives LT4 therapy. Remarkably, she also has a short stature and macrocephaly.

An overview comparing the clinical and biochemical characteristics in this family is presented in Figure 2. Comparing the phenotypes of the affected and unaffected members, there are remarkably few distinctive RTHα characteristic in this family. Constipation and mild developmental delays are seen in most but not all affected subjects. There appears to be no significant difference in TH levels, although the T_3 to rT3 ratio is higher in the affected than in the unaffected subjects. The most distinct feature of RTHα in this family is the low/borderline low hemoglobin level in six of the seven affected subjects but in none of the unaffected relatives.

Genetic and molecular analyses

Family 1

Sequencing of genomic DNA indicated that the index patient was heterozygous for a deletion of four nucleotides in exon 9 of *THRA* (c.1138-1141delTGCC), resulting in a deletion and frameshift at codon 380 and an early stop at codon 387 of TRα1 (p.C380fs387X) (Figure 1). The mutation was absent in the parents of the patient, suggesting a de novo mutation in patient 1.II.1.

Family 2

Sequence analysis showed that the index patient and his mother were heterozygous for a nucleotide substitution in *THRA* (c.1151G>A), leading to an Arg to His substitution at codon 384 in TRα1 (p.R384H) (Figure 1). The remaining members of the family did not carry the mutation.

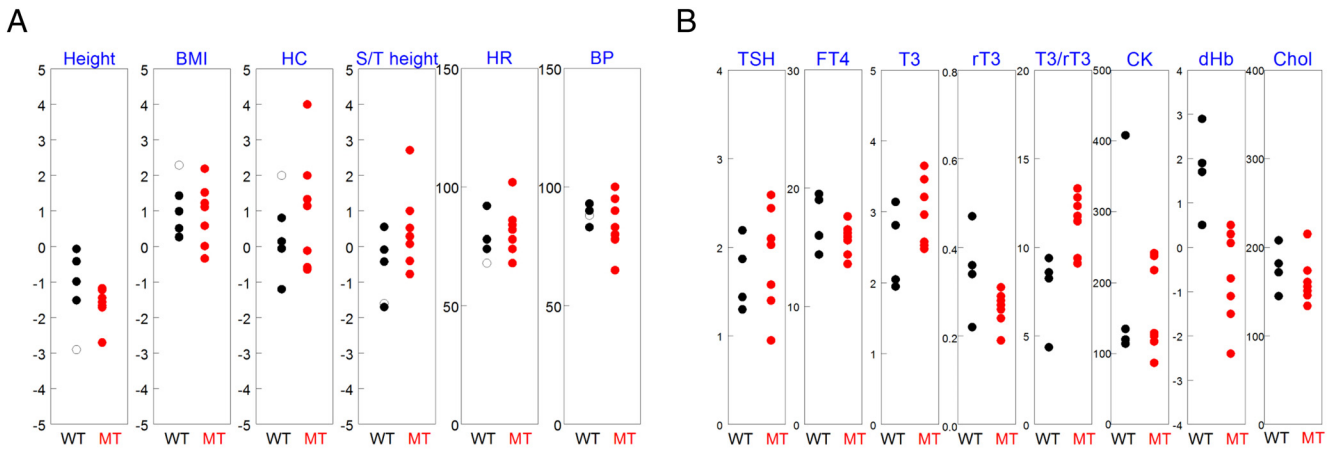

Figure 2. Clinical and biochemical parameters of family 3 (A263S TRα). A, The height, BMI, head circumference (HC), and sitting to total height (S/T height) of both affected (MT) and unaffected (WT) individuals are expressed in SDS values. Heart rate (HR) is expressed in beats per minute and the mean arterial blood pressure (BP) is expressed in millimeters of mercury. The open circle represents the LT4 treated hypothyroid WT case 3.I.1. B, TSH (milliunits per liter), FT4 (picomoles per liter), T_3 (nanomoles per liter), rT_3 (nanomoles per liter), T_3 to rT_3 ratio, CK (units per liter), ΔHb (hemoglobin vs the lower limit of normal, grams per deciliter), and cholesterol (milligrams per deciliter) values of both WT and MT family members are presented.

Family 3

DNA sequencing indicated that the index patient, his middle sister, mother, grandfather, maternal aunt, oldest niece, and cousin were heterozygous for a nucleotide substitution in *THRA* (c.G787T), leading to an Ala to Ser substitution at codon 263 (p.A263S), common to both TRα1 and TRα2 (Figure 1). The deceased member of this family (3.II.5) was not analyzed for *THRA* mutations; the remaining members were negative.

All three mutations have not been detected in public databases.

Functional analysis of the mutant receptors

To assess the effects of the mutations on the transcriptional activity of TRα1, luciferase assays were performed. In addition, the expression of all the receptor proteins was confirmed by Western blotting with an antibody against the N-terminal Flag epitope tag added to the receptor constructs. The C380fs387X, R384H, and A263S mutant receptors showed similar expression levels as WT TRα1 (Figure 3).

Family 1

Analysis of JEG3 cells cotransfected with WT or mutant TRα1 and a DR+4 TRE-luciferase reporter gene showed negligible activation of the C380fs387X mutant by up to 100 nM T_3 in contrast to marked activation of WT TRα1 (EC_{50} 0.4 nM T_3). When coexpressed, the C380fs387X mutant suppressed WT TRα1 function in a dominant-negative manner (Figure 3).

Family 2

The R384H and A263S mutations showed much milder effects on the function of TRα1. At concentrations of 1–10 nM T_3, the R384H mutant TRα1 showed a reduced transcriptional activity, but at higher T_3 levels, the R384H mutant approached the activity of WT TRα1. EC_{50} values were 22-fold higher for the R384H mutant than for WT TRα1 (8.7 nM vs 0.4 nM T_3; $P < .05$). Cotransfection of WT TRα1 with the R384H mutant did result in a moderate dominant-negative effect (Figure 3).

Family 3

The biological activity of the A263S mutant was comparable with that of WT TRα1 at most T_3 concentrations, except at the lowest concentration (0.1 nM) T_3 in which WT TRa1 was stimulated 3.6-fold and the A263A mutant 1.5-fold. EC_{50} values of A263S mutant and WT TRα1 scarcely differed (0.83 nM vs 0.33 nM T_3; $P < .05$). Coexpression with the A263S mutant hardly changed the function of WT TRα1, except that 0.1 nM T_3 produced an intermediate response compared with cells transfected with WT TRa1 alone or the A263S mutant alone (Figure 3). To investigate the biological activity of TRα2 and its possible interaction with TRα1, we cotransfected cells with WT TRα1 together with WT TRa2 or the A263S mutant in various ratios (1:1–50). Independent of the T_3 concentration, WT and A263S mutant TRα2 showed neither transcriptional activity nor dominant-negative activity toward WT TRα1 when overexpressed (Supplemental Figure 2).

Discussion

In the current study, we describe a total of 10 RTHα patients with three different mutations in TRα. This large case series enables us to report on the diversity in the phe-

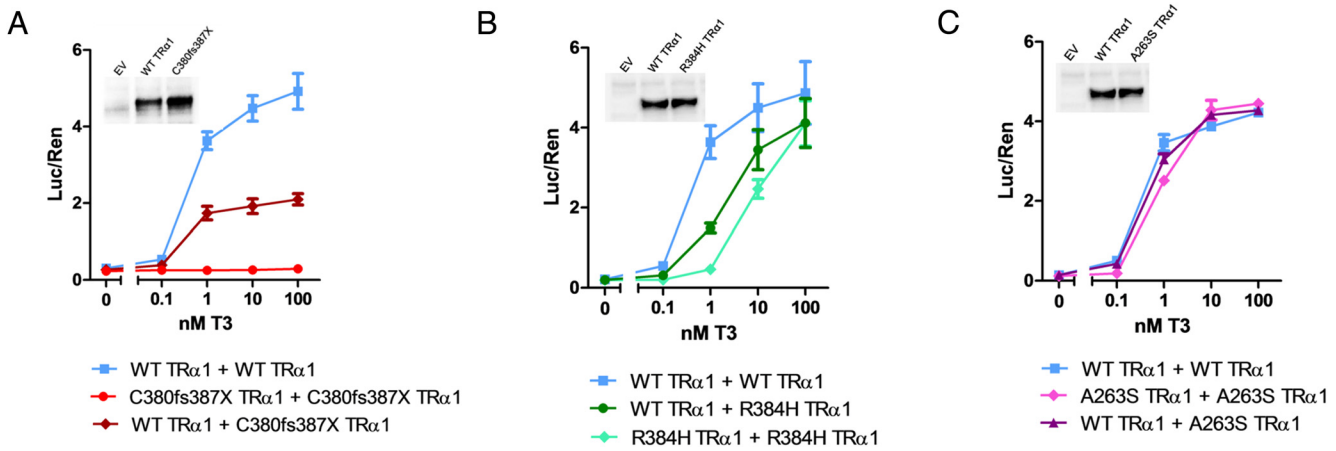

Figure 3. Functional analysis of the C380fs387X, R384H, and A263S TRα1 mutants. A, The transcriptional activity of WT and mutant TRα1 was tested in JEG3 cells transfected with a T_3-dependent promoter-reporter construct. The C380fs387X mutant had a negligible affinity for T_3 compared with WT TRα1 at all T_3 concentrations. When coexpressed, the C380fs387X mutant suppressed WT TRα1 function in a dominant-negative manner. Results are the means ± SEM of three to five experiments. B, At concentrations of 1–10 nM T_3, the R384H mutant showed a reduced transcriptional activity, but at higher T_3 levels, the maximal transcriptional activity was similar to WT TRα1. Cotransfection of WT TRα1 with the R384H mutant resulted in a moderate dominant-negative effect. C, The A263S mutant showed a comparable biological activity with WT TRα1, and coexpression of the A263S mutant hardly changed the function of WT TRα1. Luc, luciferase; Ren, Renilla.

notype of patients with different mutations and compare the phenotypes of both affected and nonaffected individuals from a family with the same mutation.

So far, four patients from three families have been described with frame-shift mutations in the C-terminal domain of TRα1: the F397fs406X mutation found in two patients from the same family by van Mullem et al (14, 15), the A382fs388X mutation found in one patient by Moran et al (12), and the C380fs387X mutation in patient 1.II.1 in the present study. Although only three frame-shift mutations have been identified so far, the more upstream the shift of these mutations occurs, the more severe the clinical phenotype appears to become. The father and daughter with the most downstream F397fs406X mutation showed marked short stature and mild delay of motor and mental development but had normal intelligence quotient (14, 15). They had been treated with LT4 from the age of 6 and 42 years, respectively. The 45-year-old female with the A382fs388X mutation, who had been treated with LT4 from the age of 3 months, presented with more severe cognitive impairment and constipation, but she was still able to walk and talk and had only mild short stature (12). Patient 1.II.1 with the most upstream C380fs387X mutation has a more severe phenotype than the above cases. Late initiation of LT4 treatment at 2.5 years of age cannot be the only cause for her global developmental delay because LT4 treatment of the patients with the F397fs406X mutation was started even much later (14, 15). Also, growth did not ameliorate with LT4 treatment, and cardiac and renal problems that may reflect tissue hypothyroidism developed during treatment. All three frame-shift mutations result in a clear dominant-negative effect on WT TRα1 in in vitro assays, explaining the marked pathogenic activity of these heterozygous mutations in the patients. At present it is unknown whether this dominant-negative activity increases with the more upstream location of the frameshift mutation.

So far, three nonsense *THRA* mutations have been reported. Bochukova et al (10) found an E403X mutation in a 6-year-old girl with developmental delay, clumsiness, severe constipation, disproportionate short stature, macrocephaly, and femoral epiphyseal dysgenesis. Tylki-Szymanska et al (13) reported the same E403X mutation in a 15-year-old female who demonstrated similar features without femoral epiphyseal dysgenesis. Similar to the patients with frameshift *THRA* mutations, the authors also found a more upstream mutation (C392X) leading to a more severe phenotype (13). There were no nonsense mutations in our case series. Different missense *THRA* mutations have previously been reported in RTHα patients with milder clinical phenotypes (11, 13, 17), and such mutations were also identified in families 2 and 3 in the present study. We report for the first time that spontaneous conception and eventless pregnancy can occur in untreated females with RTHα (2.II.1, 3.II.1, and, 3.II.3) despite varying signs of hypothyroidism. Furthermore, it is noteworthy that case 2.II.1 had a similar and even more remarkable delay in developmental milestones compared with her affected son (patient 2.III.1). However, as an adult, she had a milder intellectual deficit, no cardiac problems, and normal FT3, FT4, and TSH levels, suggesting amelioration of the clinical phenotype with time. Similar observations were made in a mouse model with a heterozygous TRα1 mutation at the same position as in family 2, ie, R384C (33, 34). These mice showed severe but transient impairment of postnatal development and growth. Alterations in TH levels were also evident only during the juvenile period. The mechanisms underlying the amelioration of deficits caused by TRα1 mutations with age are unknown.

The third family is the largest pedigree with RTHα reported so far and includes patients with the mildest phenotype in terms of growth, body habitus, and cognitive functions as well as the oldest untreated patient (3.I.2). This large family allowed us to observe the heterogeneous clinical consequences of the same THRA mutation in a single family, which is very similar to what has been described for RTHβ (2, 35). Both affected and nonaffected family members showed appreciable variation in the clinical and biochemical characteristics (Figure 2). Overall, patients with the A263S TRα1 mutation differed from healthy individuals by a higher serum T_3 to rT3 ratio and lower hemoglobin levels. Affected individuals also displayed a shorter body stature in combination with a larger head circumference, although there was a large overlap with the unaffected subjects.

The A263S mutation in this family is located at the same location as the A263V mutation found recently in a 60-year-old woman and her two young adult sons with RTHα (11). The A263V mutation resulted in poor linear growth, delayed developmental milestones, constipation, and macroglossia during infancy, and they had been treated with LT4 since 2–3 years of age (11). Their final height and body habitus were normal but all had increased head circumference. Two of them had numerous skin tags, whereas only one skin tag was present in a single individual in our series (patient 3.I.2). The functional analysis of the A263V mutant also indicated a greater defect in transcriptional activity of TRα1 than that induced by the A263S mutation (11), in further support of some degree of genotype-phenotype correlation for *THRA* mutations.

The Ala263 mutations are present in both TRα1 and TRα2. In line with previous studies of patients with the A263V or D211G (also affecting TRα1 and TRα2) mu-

tation, we did not find any evidence for additional abnormalities caused by consequences of the mutation on TRα2 (11, 17). In vitro studies did not provide any evidence for a dominant-negative effect of WT or mutant TRα2 over TRα1, in agreement with findings that the TRα2 protein is located in the cytoplasm and thus cannot interfere with the interaction of TRα1 with genomic elements and other nuclear factors (36, 37).

The A263S mutation in this family and the A263V mutation mentioned above (11) are present at the corresponding position as a mutation in *THRB* (A317V) recently identified in a family with RTHβ (38). It is also of interest to note that the R384H and R384C mutations have been identified repeatedly at the corresponding positions R438H and R438C, respectively, in *THRB* in patients with RTHβ (39), suggesting that this is also a hot spot for mutations in RTHα. As in RTHβ, the identified mutations in TRα are mostly located within three CpG-rich hot spots in the ligand-binding domain of the receptor. Considering the strong homology between the TRα1 and TRβ1 receptors and the comparable localization pattern of mutations in both receptors, the incidence of RTHα could be similar to that of RTHβ (1: 40 000) (2).

Despite T_3 resistance at the receptor level, FT3 levels are not always high. In all previously reported RTHα patients, FT4 levels were low or at the lower limit of normal. In the current study, we observed that FT4 levels were initially low in the index cases but increased into the normal range during follow-up. Accordingly, none of the affected adults had low serum FT4, suggesting attenuation in time. If rT3 levels were measured in previous cases, they were all low, which may be explained by lower precursor FT4 levels and the down-regulation of type 3 deiodinase activity. In the present study, pretreatment rT3 levels were in the normal range except for two moderately affected patients (2.II.1, 3.III.1). TSH levels were normal in all patients with *THRA* mutations, including our cases, except for the 45-year-old female patient reported by Moran et al (12), who showed a slightly increased TSH of 5.8 mU/L (N 0.35–5.5) after stopping LT4 after long-term treatment.

Despite the fact that (usually normocytic normochromic) anemia is not present in all RTHα patients, it seems to be a frequently appearing characteristic. Several studies in humans have indicated an association between hypothyroidism and anemia (40, 41). In addition, data from animal models point toward an important role for TRα in erythropoiesis (42–45). This supports the hypothesis that mutations in TRα contribute to the pathogenesis of anemia seen in RTHα patients.

In conclusion, this study shows that the clinical consequences of mutations in *THRA* are heterogeneous. This is the case for patients with different TRα mutations but also for family members with the same mutation. Common characteristics in almost all patients comprise a delay in reaching developmental milestones, constipation, and anemia. Growth retardation and macrocephaly are less consistent. The clinical phenotype varies from severe to very mild, in part depending on the type and location of the mutation. Therefore, mutations in *THRA* should be suspected in subjects with even mild developmental delays, anemia, and a high serum T_3 to rT_3 ratio.

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