

Supplemental Table 1. Thyroid function test measured after PEG precipitation in genuine primary hyperthyroidism and normal controls

	fT4		fT3	
	Direct measure	PEG precipitation	Direct measure	PEG precipitation
Hyperthyroid 1	52.9	49.0	14.4	9.2
Hyperthyroid 2	35.8	34.4	11.5	7.9
Hyperthyroid 3	43.2	40.1	11.5	7.1
Hyperthyroid 4	61.0	52.6	23.6	17.7
Hyperthyroid 5	39.3	38.6	11.1	8.74
Hyperthyroid 6	36.0	30.6	8.6	6.01
Control 1	13.6	14	5.3	4.8
Control 2	13.8	12.4	3.7	3.37
Control 3	14.9	13.0	5.6	4.51
Reference range	11.5-24.5		2.9-7.1	

Supplemental Table 2. Clinical and genetic features of patients with congenital hypothyroidism or non-autoimmune hypothyroidism referred to exclude associated RTH β because of borderline high fT4 and high TSH

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age(years)/sex	2/Female	10/Female	7/Female	45/Female	52/Male	28/Male
TSH (mU/L)	18.0	8.8	7.87-2.13	4.53	6.5-7.3	9.02
fT4 (pmol/L)	23.7	25.9	25.7 -25.1	21.8	20.0-21.1	20.4
fT3 (pmol/L)	6.4	–	–	4.6	5.5	7.0
Genetic Findings	Wild-type	Wild-type	<i>DUOX2</i> (p.K1474E, p.R1510H); <i>TG</i> (p.E1133K, p.R1136Q)	<i>TSHR</i> (p.E34K); <i>TPO</i> (p.P906L)	<i>TSHR</i> (p.R109G)	<i>TSHR</i> (p.Y444H)
Thyroid phenotype	Ectopic (sublingual)	Thyroid agenesis	Ectopic (sublingual)	heterogenous echotexture normally sized	Normal, normoechoic; negative TPO- and Tg-Ab	Normal, normoechoic; negative TPO- and Tg-Ab
Thyroid autoantibodies (Tg- /TPO-Ab)	-	-	-	Tg-Ab negative TPO-Ab borderline	Negative	Negative
Levothyroxine at the time of referral	Yes	Yes	Yes	Yes	No	No

reference ranges: TSH: 0.4-4.0 mU/L; fT4: 11.5-24.5 pmol/l; fT3 adult 2.9-7.1 pmol/l; children 3.8-8.6 pmol/l

Supplemental Table 3. Thyroid function test measured every 30-90 days during methimazole therapy of case 2

	baseline	FU1	FU2	FU3	FU4	FU 5	FU 6	FU 7	Reference range
TSH mU/L	0.1	0.27	2.9	2.6	4.8	3.2	7	5.8	0.270-4.5
FT4 pmol/L	50	46	25	35	20.9	29.6	19.6	22.1	9-19
FT3 pmol/L	NA	12.7	11.4	12	11	15.2	13.8	13.5	3.9-6.8
Methimazole (mg/die)	-	30	20	10	15	10	15	15	

NA not available, FU: follow-up

Supplemental Table 4. TRH test results of case 2

		baseline	15 minutes	30 minutes
TSH mU/L	untreated serum	0.1	0.27	2.9
	PEG precipitation	0.83	3.24	4.42
PRL mcg/L	untreated serum	1.9	11.1	10.8
	PEG precipitation	5.36	31.8	29.6

Supplemental Table 5. Hormonal Profile by Tandem Mass Spectrometry in Patient 5

Hormone	Results (ng/mL)	Male reference interval (ng/mL)
Cortisone	16.5	Not available
Cortisol	128.7	45.7 - 199.4
21-Deoxycortisol	< 0.078	<0.078
Corticosterone	2.33	0.46 - 12.60
11-Deoxycortisol	0.724	0.086 - 1.094
Androstenedione	0.883	0.262 - 1.263
11-Deoxycorticosterone	< 0.078	<0.146
Testosterone	4.319	2.82 - 8.18
17-Hydroxyprogesterone	0.383	0.415 - 2.542
Dehydroepiandrosterone	3.61	1.40 - 14.28
Progesterone	< 0.049	<0.189

Supplemental Figure 1. THRβ mutations identified in RTHβ patients

