Supplementary material to

Long-term efficacy of T3 analogue Triac in children and adults with MCT8 deficiency: a real-life retrospective cohort study

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Supplementary methods

Analyses of thyroid function tests and biochemical parameters

Serum total T3 concentrations were measured by Vitros ECI technology (Ortho-Clinical Diagnostics, Beerse, Belgium) until April 12 2019 and afterwards by Lumipulse G1200 (Fujirebio Inc., Ghent, Belgium), and by Immulite 2000 XPi (Siemens Healthcare, The Hague, The Netherlands). Free T4, total T4 and TSH concentrations were measured by Vitros ECI technology (Ortho-Clinical Diagnostics, Beerse, Belgium) until April 12 2019 and afterwards by Lumpulse G1200 (Fujirebio Inc., Ghent, Belgium). Sex hormone-binding globulin (SHBG) was measured using an immunometric method (Immulite 2000 XPi (Siemens Healthcare, The Hague, The Netherlands)). Creatine kinase (CK) and creatinine were measured by Cobas 8000 (Roche Diagnostics, Almere, The Netherlands).

To account for any interference of Triac in the measurement of serum T3 concentrations, we used an algorithm based on the different levels of cross-reactivity of Triac in two T3 assays, as reported before (1). To minimize the interference of Triac in the T3 assays, parents were instructed to have blood samples collected in the morning before the administration of the morning Triac dose. This procedure ensured a minimum washout time of ~8 hours. With a half-life time of 6.5 hours, Triac concentrations in the blood were expected at their lowest levels (2, 3).

In case of suspected endogenous antibody interference in the T3 Vitros assay at baseline measurement (defined as a difference with other assays of more than 5 nmol/L) (4), T3 Lumipulse or T3 radioimmunoassay (RIA) measurement was used for analyses.

Analyses of anthropometric parameters and heart rate

Body weight- and height-for-age and weight-for-height Z scores were calculated using the TNO growth calculator (5) and heart rate-for-age Z scores were calculated using the Boston Z score calculator (6). Weight-for-age and height-for-age Z scores were compared to the available natural history data obtained in a cohort of Triac-naïve patients (7). The difference to the natural history curve was

determined for each subject at indicated time-points and used for comparison. Using this strategy, the deterioration of these parameters in untreated patients was fully considered. Given the scarcity of natural history data in subjects aged above 18 years, subjects older than 18 years at baseline were excluded from analyses. Tanner stages of sexual maturation were determined by each patient's physician.

In addition to statistical analyses, changes in body weight- and height-for-age compared to the natural history reference line and heart rate-for-age were descriptively analyzed. Increase of body weight- and height-for-age compared to the natural history reference line and heart rate-for-age was defined as a change from baseline to last visit of >0.25 SDs. Stabilization of body weight- and height-for-age compared to the natural history reference line and heart rate-for-age was defined as a change from baseline to last visit of 0.25 to -0.25 SDs. Decrease of body weight- and height-for-age compared to the natural history reference line and heart rate-for-age was defined as a change from baseline to last visit of <-0.25 SDs. These cut-off values were chosen as they were deemed clinically relevant by the lead investigators (FSvG, SG, WEV). Changes in Tanner stages of sexual maturation were descriptively analyzed.

Safety

The data capture monitoring system included the following pre-defined adverse events during follow-up:

- Diarrhea
- Vomiting
- Sweating
- Shortness of breath
- Skin rash
- Anxiety
- Other

Other safety end points were embedded in the clinical outcomes such as heart rate and body weight. In addition, the occurrence of hospital admission and its relationship with Triac treatment were monitored during follow-up.

Figure S1: Study design

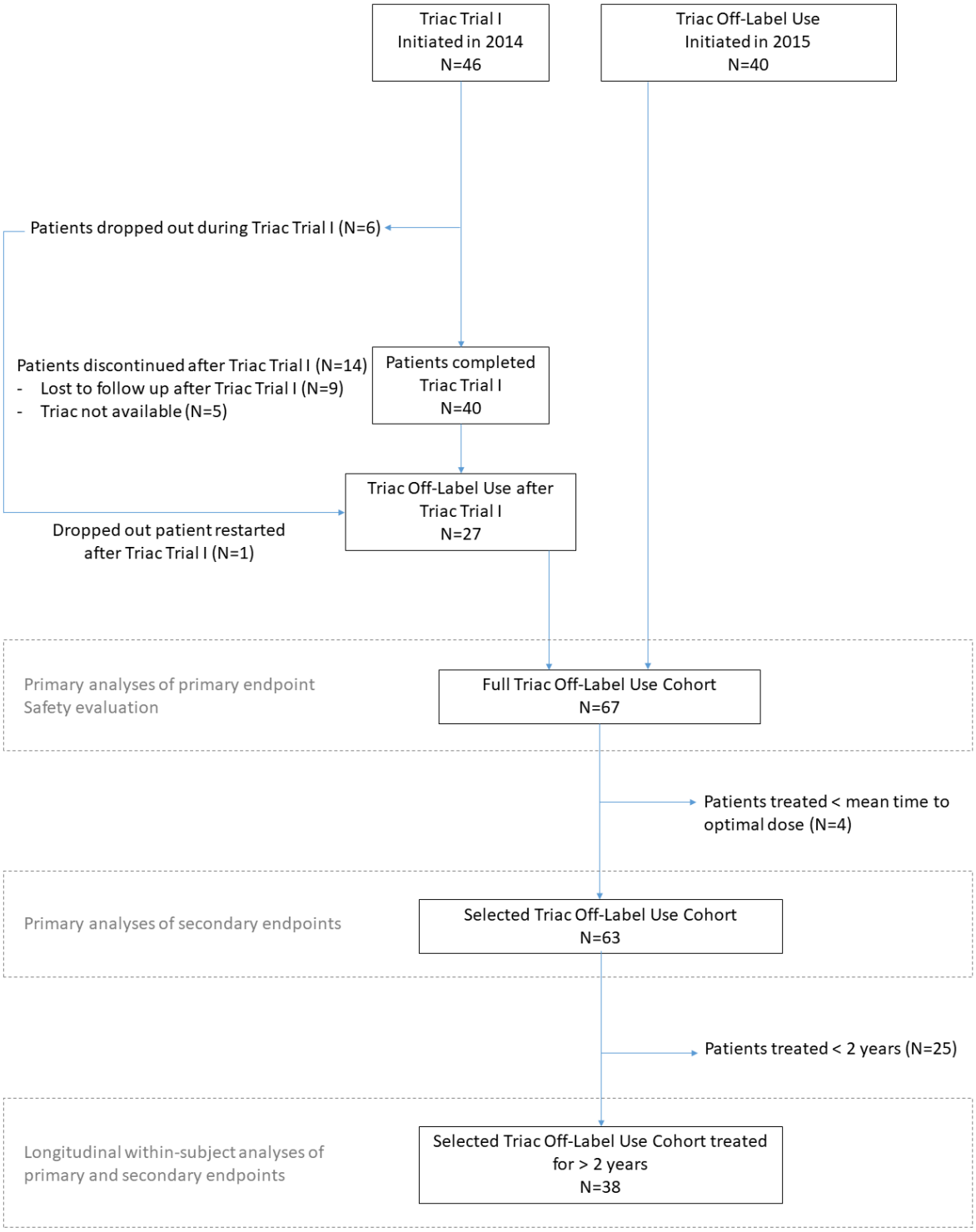


Figure S1: Outline of the study. Grey dashed boxes indicate the cohorts selected for analyses. All patients who concluded Triac Trial I were offered to continue Triac treatment on off-label use basis when possible. Continuation of treatment on off-label use basis was not possible in all countries mostly due to regulatory difficulties (applicable for five patients). During the Triac Trial I, newly identified

families who pursued Triac treatment were informed about the existence of Triac Trial I. However, for 12 patients participation was not possible due to the absence of a hub center in their country and 28 patients were identified after the recruitment phase.

Figure S2: Distribution of treated patients around the world

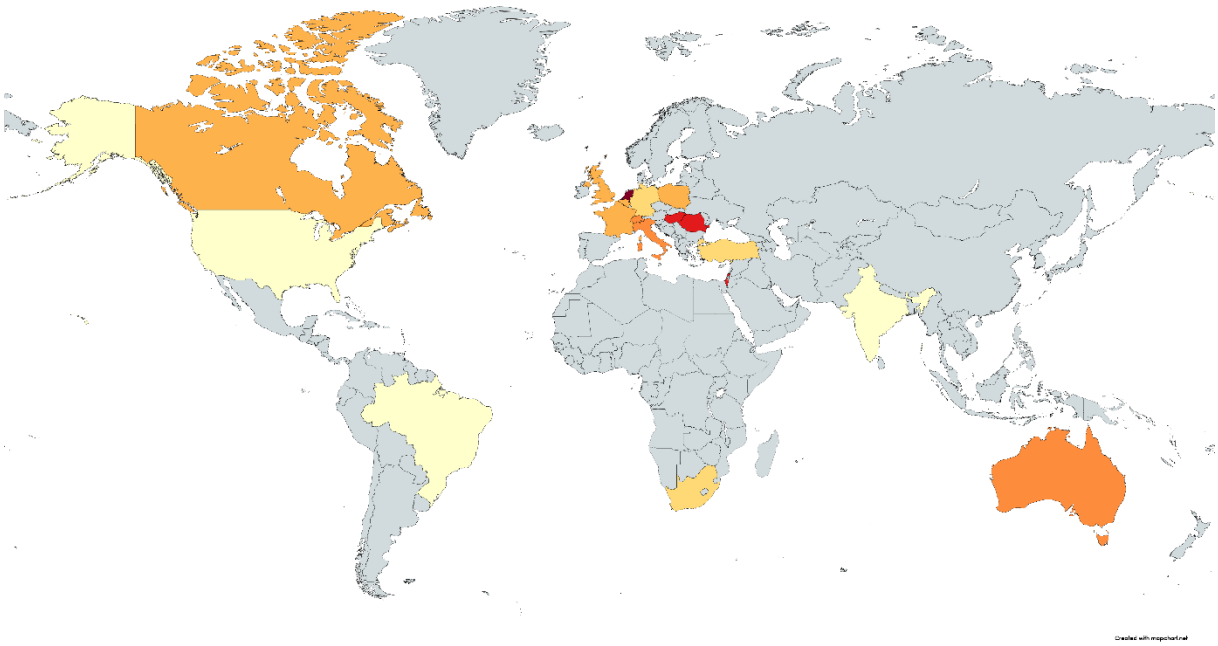
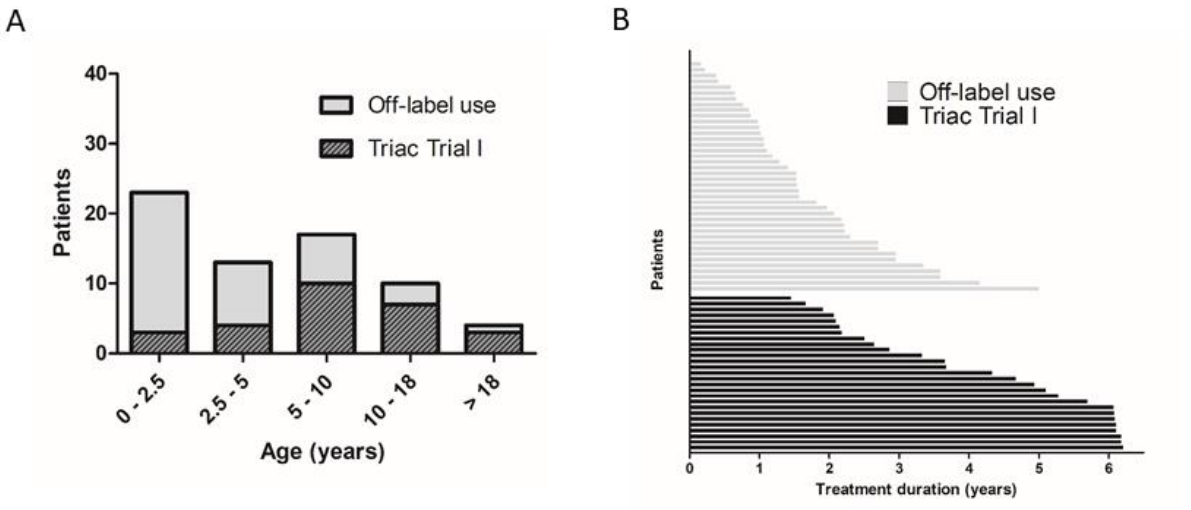


Figure S2: Light yellow indicates <0.1 treated patients per 10 million residents; yellow indicates 0.1 – 0.5 treated patients per 10 million residents; light orange indicates 0.5 – 1 treated patients per 10 million residents; orange indicates 1 – 2 treated patients per 10 million residents; red indicates 2 – 5 treated patients per 10 million residents; dark red indicates > 5 treated patients per 10 million residents (8, 9).

88 **Figure S3: Cohort characteristics**



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90 **Figure S3:** Characteristics of the combined cohort, divided into patients originally enrolled in Triac Trial
91 I (Triac Trial I) and patients who were directly treated on off-label use basis (Off-label use). (A) Age
92 distribution of treated patients; (B) Treatment exposure of patients, shown in years.

Figure S4: Timing of measurement of outcomes

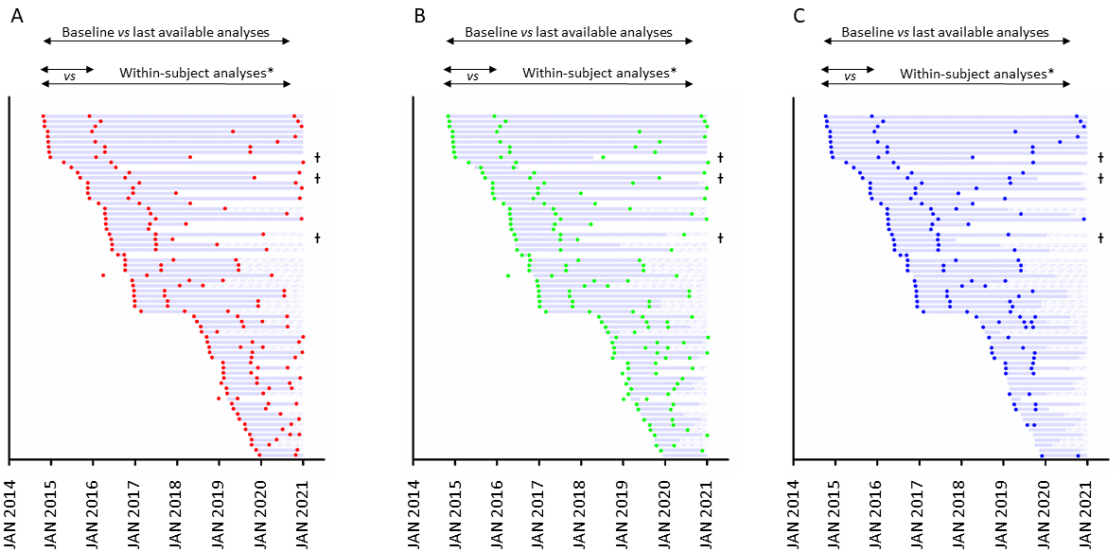

Figure S4: Timing of baseline, interim and last available measurement of outcomes for each patient. (A) Thyroid function tests; (B) Anthropometric outcomes and heart rate; (C) Peripheral markers of thyroid hormone action. Individual points indicate the timing of outcome measurement at baseline, after one year of treatment and at the last visit (or last available). Blue bars represent the treatment period of each patient (based on the last serum control in the central lab, except for patients followed in the Erasmus Medical Center); dashed light blue bars indicate continuation of treatment up to January 1th 2021 (81% of patients). In 9 patients, the last available measurements of the different anthropometric outcomes and heart rate were captured at different moments. *Longitudinal within-subject analyses were performed in patients who were treated >2 years. †Patient deceased. Abbreviations: JAN=January.

Figure S5: Change from baseline to last visit in height-for-age

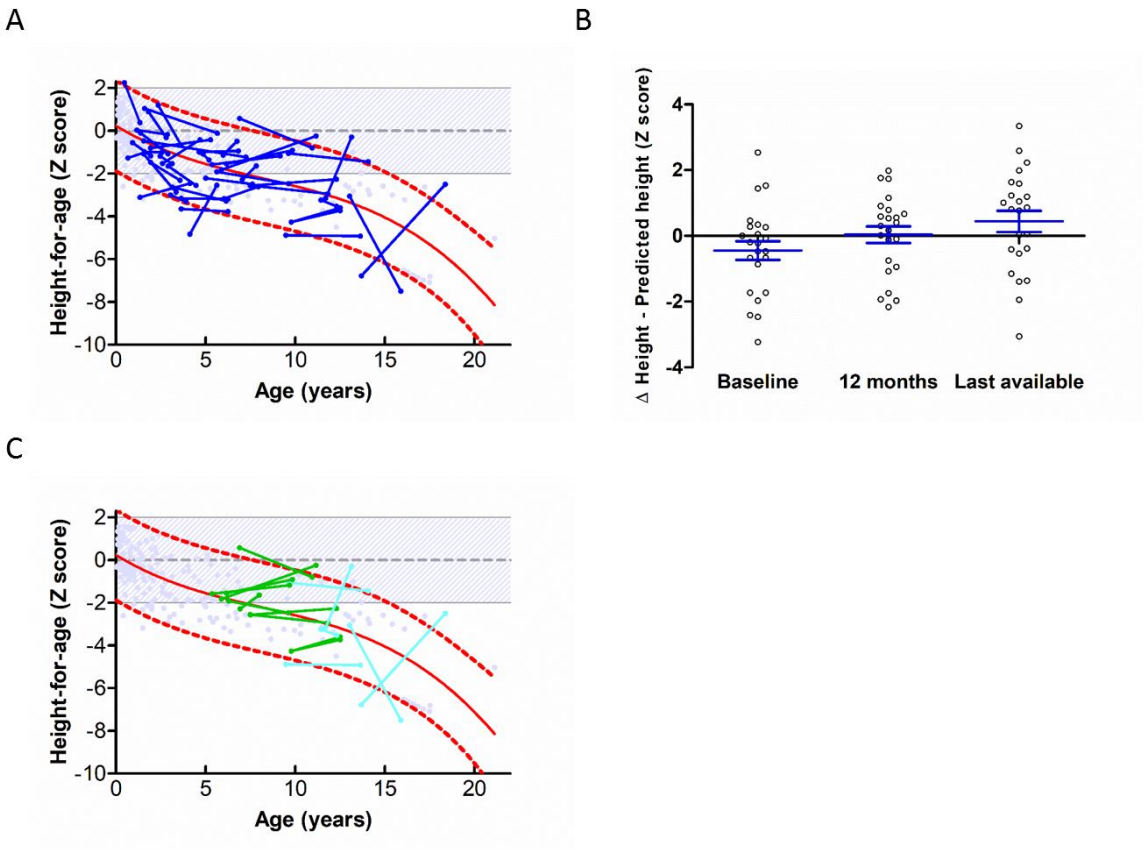

Figure S5: Panel A shows changes in body height-for-age between baseline and last visit (blue dots and lines; n=43); the natural history of untreated patients with MCT8 deficiency is depicted in grey dots with the historical reference line in red (with the 95% error band in dashed lines), based on a historical control group (7). Panel B shows the change in difference between the body height-for-age Z-score and the expected Z-score based on the natural history data on the corresponding age from baseline to one year and last available visit (longitudinal within-subject analyses; n=23). Panel C shows changes in body height-for-age between baseline and last visit (dots and lines; n=16) of patients who were in pubertal age (8-18 years) during treatment; green lines indicate patients in pre-pubertal state, light blue lines indicate patients in puberty. The natural history of untreated patients with MCT8 deficiency is depicted in grey dots with the historical reference line in red (with the 95% error band in dashed lines), based on a historical control group (7). Median treatment duration was 2.2 years (IQR 1.5 – 3.9

118 years) for analyses of secondary outcomes (panel A) and 3.6 years (IQR 2.5 – 5.2 years) for longitudinal
119 within-subject analyses (panel B). Body height-for-age Z scores were calculated using TNO growth
120 calculator.

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Figure S6: Change from baseline to last visit in serum TSH, free T4 and T4 concentrations

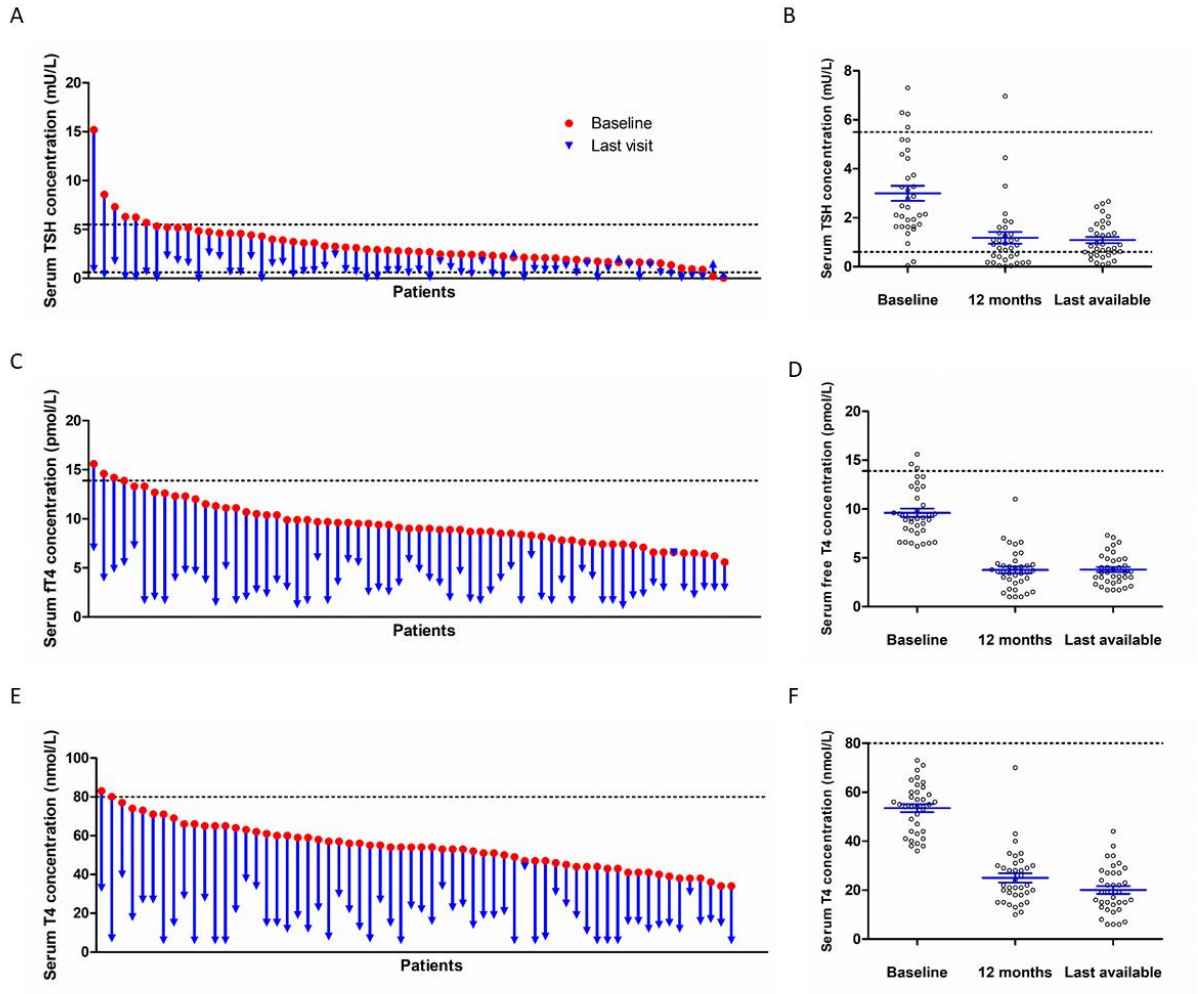

Figure S6: Panel A shows changes in serum concentrations of TSH between baseline and last available follow-up visit on treatment with Triac (n=61). Panel B shows the change in serum TSH concentrations from baseline to one year and last available visit (longitudinal within-subject analyses; n=34; for clarity, data are depicted as non-transformed in panels A and B). Panel C shows changes in serum concentrations of free T4 between baseline and last available follow-up visit on treatment with Triac (n=63). Panel D shows the change in serum free T4 concentrations from baseline to one year and last available visit (longitudinal within-subject analyses; n=36). Panel E shows changes in serum concentrations of T4 between baseline and last available control visit on treatment with Triac (n=62). Panel F shows the change in serum T4 concentrations from baseline to one year and last available visit (longitudinal within-subject analyses; n=36). Median treatment duration was 2.2 years (IQR 1.5 – 3.9

years) for analyses of secondary outcomes (panels A, C and E) and 3.6 years (IQR 2.5 – 5.2 years) for longitudinal within-subject analyses (panels B, D and F). Black dashed lines represent the reference intervals (for the median baseline age). Red dots represent baseline measurement and blue arrows represent the last available measurement in panels A, C and E. Grey dots represent measurements in the individual patients in panels B, D and F; means and SEM are displayed in blue. Results of analyses after stratification based on treatment duration are provided in **supplementary table 1**. Abbreviations: TSH=thyroid-stimulating hormone. T4=thyroxine.

Figure S7: Change from baseline to last visit in Tanner stage in patients between 8 and 18 years during treatment

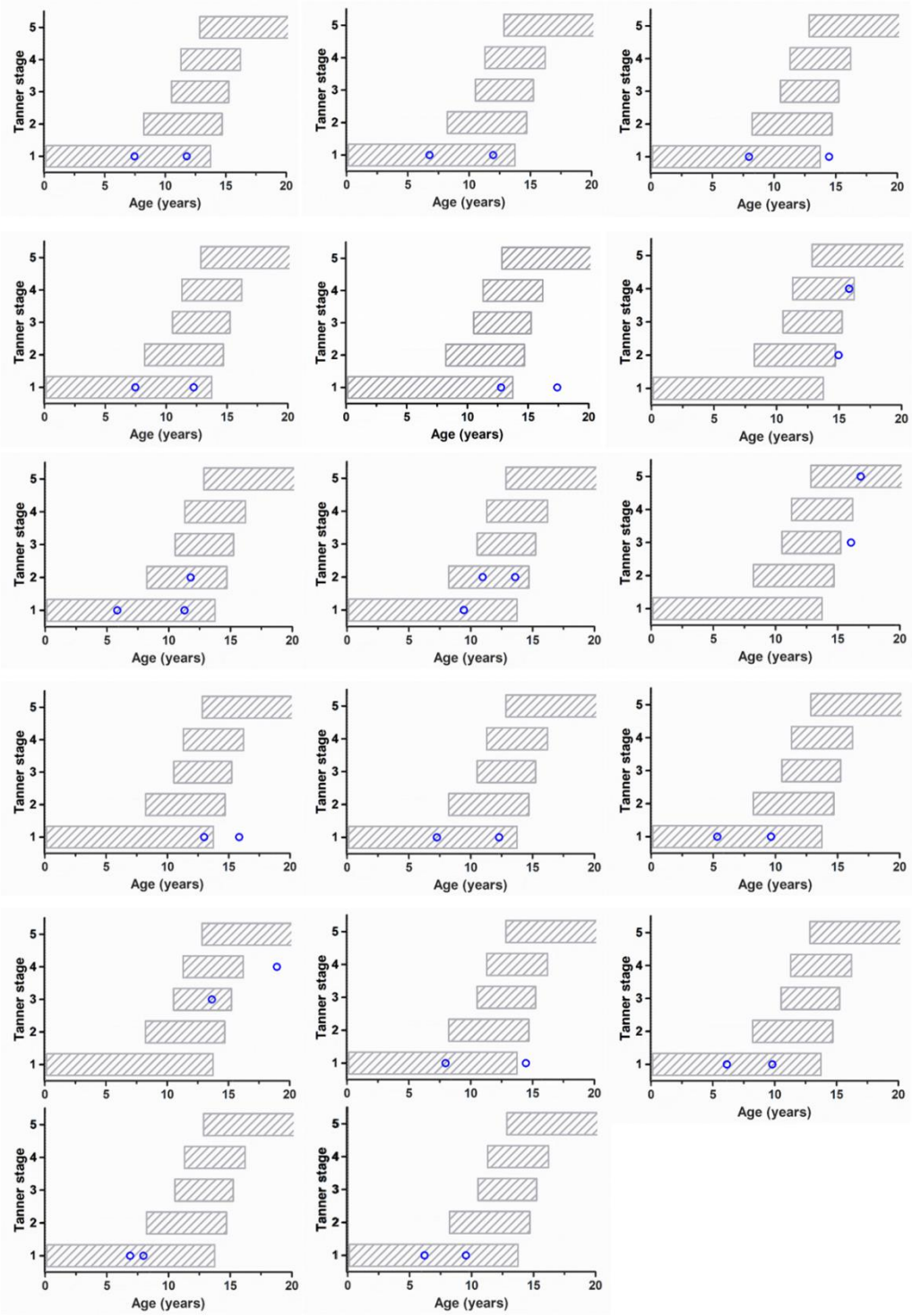

Figure S7: Changes in Tanner stage on treatment with Triac in patients between 8 and 18 years during treatment. Each graph represents an individual patient (n=17; complete information not available for 7 patients). Blue dots represent individual measurement. Grey bars represent the normal ranges (based on (10), with normal ranges determined as median ± 2SD).

Table S1: Mean changes from baseline to last visit in primary and secondary outcomes after stratification based on treatment duration

Treatment duration (years)	< 1 n=7	≥ 1 – 3 n=34	≥ 3 – 5 n=10	≥ 5 n=12	P value
Primary outcome					
T3 (nmol/L)	-2.68 (1.01)	-3.11 (1.46)	-3.19 (0.73)	-2.38 (1.20)	0.2715
Secondary outcomes					
Anthropometric parameters and heart rate					
Body weight (kg)	3.08 (2.87)	3.79 (2.74)	6.56 (7.78)	11.15 (6.47)	
Weight-for-age Z score	0.55 (1.73)	0.28 (1.21)	-0.33 (1.46)	0.14 (1.40)	0.5717
Δ Weight-for-age – predicted weight-for-age Z score	0.85 (1.65)	0.76 (1.11)	0.36 (1.49)	0.88 (1.89)	0.8464
Height (cm)	8 (6)	10 (7)	22 (14)	28 (9)	
Height-for-age Z score	0.21 (0.47)	-0.30 (1.42)	0.38 (1.51)	-0.14 (0.97)	0.6006
Δ Height-for-age – predicted height-for-age Z score	0.47 (0.47)	0.18 (1.35)	1.44 (2.05)	1.03 (0.90)	0.1506
Heart rate (bpm)	-2 (24)	-13 (24)	-29 (21)	-24 (21)	0.1274
Heart rate-for-age Z score	0.00 (1.27)	-0.42 (1.13)	-1.31 (1.05)	-1.01 (1.24)	0.0991
Thyroid function tests					
TSH (mU/L)*	-2.15 (1.51)	-2.90 (2.79)	-1.69 (1.69)	-1.69 (1.69)	0.0981
Free T4 (pmol/L)	-5.9 (2.1)	-6.7 (2.3)	-6.6 (2.5)	-3.9 (1.8)	0.0055
T4 (nmol/L)	-40 (18)	-36 (13)	-41 (14)	-30 (11)	0.2325
Peripheral markers					
Sex hormone-binding globulin (nmol/L)	-1 (61)	-48 (69)	-10 (88)	-39 (58)	0.3788
Creatinine (µmol/L)	8 (1)	5 (5)	10 (5)	10 (5)	0.0403
Creatine kinase (U/L)*	56 (36)	33 (86)	4 (29)	-13 (129)	0.5551
All outcomes were assessed in all patients who received Triac treatment longer than the mean time to optimal dose (5.0 months). Data are represented as mean (SD). Abbreviations: T3=tri-iodothyronine. TSH=thyroid-stimulating hormone. T4=thyroxine. *TSH and creatine kinase concentrations were log-transformed to ensure a normal distribution before one-way ANOVAs were done (non-transformed means [SDs] are presented for the sake of interpretability).					

Table S2: Mean changes from baseline to one year and last visit in primary and secondary outcomes (longitudinal within-subject analyses)

	Baseline mean (SD)	One year mean (SD)	Mean change from baseline (95% CI)	Last visit mean (SD)	Mean change from baseline (95% CI)	P value *
Primary outcome						
T3 (nmol/L; n=36)	4.60 (0.99)	1.80 (0.62)	-2.81 (-3.27 to -2.34)	1.70 (0.60)	-2.90 (-3.37 to -2.44)	<0.0001
Secondary outcomes						
Anthropometric parameters and heart rate						
Body weight (kg; n=34)	19.4 (12.5)	21.8 (12.1)	2.4 (0.2 to 4.6)	27.1 (15.1)	7.7 (5.6 to 9.9)	
Weight-for-age Z score (n=34)	-3.10 (1.96)	-2.91 (1.86)	0.18 (-0.32 to 0.68)	-2.91 (1.85)	0.19 (-0.31 to 0.69)	0.4579
Δ Weight-for-age – predicted weight-for-age Z score (n=32)	0.03 (1.92)	0.44 (1.92)	0.42 (-0.13 to 0.96)	0.88 (2.02)	0.86 (0.31 to 1.40)	0.0039
Height (cm; n=24)	108 (21)	116 (21)	8 (3 to 13)	127 (20)	19 (14 to 25)	
Height-for-age Z score (n=24)	-2.11 (1.79)	-1.92 (1.41)	0.19 (-0.34 to 0.78)	-2.13 (1.75)	-0.03 (-0.61 to 0.56)	0.9335
Δ Height-for-age – predicted height-for-age Z score (n=23)	-0.45 (1.37)	0.04 (1.21)	0.48 (-0.18 to 1.14)	1.44 (1.56)	0.89 (0.23 to 1.55)	0.0178
Heart rate (bpm; n=19)	114 (25)	103 (17)	-11 (-24 to 1)	95 (17)	-20 (33 to 7)	0.0036
Heart rate-for-age Z score (n=19)	1.74 (1.08)	1.36 (0.81)	-0.38 (-1.01 to 0.25)	1.03 (0.77)	-0.70 (-1.33 to -0.09)	0.0271
Thyroid function tests						
TSH (mU/L; n=34)†	2.99 (1.79)	1.18 (1.39)	-1.82 (-2.53 to -1.11)	1.08 (0.74)	-1.91 (-2.62 to -1.20)	<0.0001
Free T4 (pmol/L; n=36)	9.6 (2.6)	3.8 (2.1)	-5.9 (-6.9 to -4.8)	3.8 (1.6)	-5.8 (-6.9 to -4.8)	<0.0001
T4 (nmol/L; n=36)	54 (10)	25 (11)	-29 (-34 to -23)	20 (10)	-33 (-39 to -28)	<0.0001
Peripheral markers						
Sex hormone-binding globulin (nmol/L; n=22)	229 (91)	188 (72)	-40 (-74 to -7)	187 (78)	-41 (-75 to -7)	0.0084
Creatinine (µmol/L; n=19)	35 (11)	40 (15)	6 (2 to 9)	43 (14)	8 (4 to 12)	<0.0001
Creatine kinase (U/L; n=19)†	125 (121)	211 (279)	86 (-29 to 201)	135 (84)	10 (-105 to 125)	0.2579

All outcomes were assessed in all patients who received Triac treatment longer than the mean time to optimal dose (5.0 months). Data are mean. Abbreviations: T3=tri-iodothyronine. TSH=thyroid-stimulating hormone. T4=thyroxine. *Paired T-tests were used to detect significant changes from baseline to last visit. †TSH and creatine kinase concentrations were log-transformed to ensure a normal distribution before one way ANOVAs were done (non-transformed means [SDs] are presented for the sake of interpretability).

155 **Table S3: Changes from baseline to last visit in secondary outcomes on full analysis set**

	Baseline mean (SD)	Last visit mean (SD)	Mean change (95% CI)	P value
Anthropometric parameters and heart rate				
Body weight (kg; n=62)	17.2 (12.0)	22.6 (14.5)	5.4 (4.0 to 6.8)	
Weight-for-age Z score (n=62)	-2.84 (1.94)	-2.67 (1.79)	0.17 (-0.17 to 0.50)	0.3230
Δ Weight-for-age – predicted weight-for-age Z score (n=59)	-0.03 (1.84)	0.66 (1.92)	0.70 (0.35 to 1.05)	0.0002
Height (cm; n=46)	100 (21)	115 (23)	15 (11 to 18)	
Height-for-age Z score (n=46)	-1.82 (1.76)	-1.90 (1.51)	-0.09 (-0.48 to 0.31)	0.6615
Δ Height-for-age – predicted height-for-age Z score (n=45)	-0.45 (1.37)	0.11 (1.40)	0.56 (0.12 to 1.00)	0.0135
Weight-for-height Z score (n=46)	-2.09 (2.51)	-1.58 (2.43)	0.51 (-0.33 to 1.34)	0.2272
Heart rate (bpm; n=52)	114 (20)	98 (19)	-16 (-22 to -9)	<0.0001
Heart rate-for-age Z score (n=52)	1.56 (0.87)	0.96 (0.98)	-0.60 (- 0.92 to -0.27)	0.0005
Thyroid function tests				
TSH (mU/L; n=65)*	3.47 (2.37)	1.04 (0.96)	-2.43 (-2.99 to -1.86)	<0.0001
Free T4 (pmol/L; n=67)	9.6 (2.3)	3.5 (1.7)	-6.0 (-6.6 to -5.5)	<0.0001
T4 (nmol/L; n=66)	54.6 (11.9)	18.7 (10.3)	-35.8 (-39.2 to -32.5)	<0.0001
Peripheral markers				
Sex hormone-binding globulin (nmol/L; n=48)	247 (100)	212 (93)	-35 (-55 to -15)	0.0011
Creatinine (µmol/L; n=46)	32 (11)	39 (13)	7 (6 to 9)	<0.0001
Creatine kinase (U/L; n=46)*	110 (87)	128 (80)	18 (-8 to 45)	0.2166

All outcomes were assessed in all patients who received Triac treatment longer than the mean time to optimal dose (5.0 months). Data are mean. TSH=thyroid-stimulating hormone. T4=thyroxine. *TSH and creatine kinase concentrations were log-transformed to ensure a normal distribution before paired t tests were done (non-transformed means [SDs] and mean changes [95% Cis] are presented for the sake of interpretability).

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158 **Table S4: Overview of Adverse Events deemed related to Triac treatment as judged by the investigators**

	Adverse event	Age start treatment (years)	Character	Dose (µg/day)	Triac dose altered after AE?	Outcome
#1	Increased irritability	6.0	Transient	525	Yes, decreased	Resolved without sequelae
#2	Increased anxiety	13.7	Transient	1050	No	Resolved without sequelae
#3	Increased anxiety and sadness	6.3	Transient	700	No	Resolved without sequelae
#4	Increased irritability and reduced sleep	1.2	Transient, after dose increase	350	Yes, decreased	Resolved without sequelae
#5*	Increased sweating and irritability, tachycardia	15.9	Transient	700	No	Returned later during treatment (irritability), otherwise resolved without sequelae
#6*	Increased irritability and anxiety	15.9	Transient	1400	No	Returned later during treatment (anxiety), otherwise resolved without sequelae
#7*	Increased blood pressure, tachycardia and increased anxiety	15.9	Transient, after dose increase (for increased blood pressure and tachycardia)	1700	Yes, decreased	Resolved without sequelae
*These events occurred in the same patient.						

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