Early thyroxine treatment in Down syndrome and thyroid function later in life

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Abstract

Objective: The hypothalamus–pituitary–thyroid (HPT) axis set point develops during the fetal period and first two years of life. We hypothesized that thyroxine treatment during these first two years, in the context of a randomized controlled trial (RCT) in children with Down syndrome, may have influenced the HPT axis set point and may also have influenced the development of Down syndrome-associated autoimmune thyroiditis.

Methods: We included 123 children with Down syndrome 8.7 years after the end of an RCT comparing thyroxine treatment vs placebo and performed thyroid function tests and thyroid ultrasound. We analyzed TSH and FT4 concentrations in the subgroup of 71 children who were currently not on thyroid medication and had no evidence of autoimmune thyroiditis.

Results: TSH concentrations did not differ, but FT4 was significantly higher in the thyroxine-treated group than that in the placebo group (14.1 vs 13.0 pmol/L; *P* = 0.02). There was an increase in anti-TPO positivity, from 1% at age 12 months to 6% at age 24 months and 25% at age 10.7 years with a greater percentage of children with anti-TPO positivity in the placebo group (32%) compared with the thyroxine-treated group (18.5%) (*P* = 0.12). Thyroid volume at age 10.7 years (mean: 3.4 mL; range: 0.5–7.5 mL) was significantly lower (*P* < 0.01) compared with reference values (5.5 mL; range: 3–9 mL) and was similar in the thyroxine and placebo group.

Conclusion: Thyroxine treatment during the first two years of life led to a mild increase in FT4 almost 9 years later on and may point to an interesting new mechanism influencing the maturing HPT axis set point. Furthermore, there was a trend toward less development of thyroid autoimmunity in the thyroxine treatment group, suggesting a protective effect of the early thyroxine treatment. Lastly, thyroid volume was low possibly reflecting Down-specific thyroid hypoplasia.

Introduction

Down syndrome is characterized by an extra copy of chromosome 21 and is associated with various congenital malformations such as heart defects and digestive system malformations. Thyroid dysfunction is also frequent in Down syndrome, including congenital primary (or thyroidal) hypothyroidism and acquired autoimmune thyroid dysfunction (1). Besides clinically evident forms of hypothyroidism, subclinical hypothyroidism with only very mild thyrotropin (TSH) elevation is also frequently encountered in Down syndrome (1, 2, 3). As Down syndrome neonates not only have mildly elevated TSH levels but also lower T4 concentrations compared with non-Down syndrome neonates, it has been suggested that there may be an altered hypothalamic–pituitary–thyroid (HPT)
axis set point in Down syndrome or it may indicate a mild form of Down syndrome-specific congenital hypothyroidism of thyroidal origin (1, 2, 3). Based on the assumption that young children with Down syndrome as a group have a mild form of congenital thyroidal hypothyroidism, it was hypothesized that thyroxine treatment during the first two years of life could improve the psychomotor development in children with Down syndrome. Between 1999 and 2003, a randomized clinical trial (RCT) was conducted to study the effects of thyroxine treatment vs placebo during the first two years of life on psychomotor development in a cohort of children with Down syndrome (4). In this RCT, thyroxine treatment resulted in somewhat better motor development and growth compared with placebo treatment at the age of 2 years (4). As the primary outcome of this study was early mental and motor development, treatment was discontinued at the age of two years. In the Netherlands, publication of the trial results in 2005 led to a debate on whether thyroxine treatment should be started in all neonates with Down syndrome. With only modest improvements in motor development (0.7 months reduction in delay) and growth (0.9 cm height gain), but no clear positive effect on mental development, the benefits of early started treatment were considered not to outweigh the burden of daily treatment (4). In a follow-up study performed at the age of 10.7 years, no developmental improvement was found. In that same follow-up study, we had the opportunity to study the effect of thyroxine vs placebo treatment during the first two years of life on the HPT axis and the development of thyroid autoimmunity later on in life (5).

The HPT axis set point develops during the fetal period followed by slowly decreasing TSH and minimal change in FT4 concentrations in the first two years of life. After that period, the FT4 set point remains stable into adulthood (6, 7, 8). As there is evidence suggesting that environmental factors may be even more important than genetic factors in determining HPT axis set point (9, 10), we hypothesized that thyroxine treatment in the first two years of life, resulting in a clearly higher plasma FT4 concentration in the period that the HPT axis is not completely mature, may have caused an HPT axis set point change that persists later on in life.

Autoimmune thyroid disease is reported to be common in Down syndrome after the age of eight years, with a gradually increasing concentration of thyroid autoantibodies (11, 12). As early thyroxine treatment in autoimmune thyroiditis has been reported to delay the disease progression, we studied the development of thyroid autoimmunity in this cohort of very early treated children with Down syndrome (13, 14).

Finally, the follow-up study enabled us to measure thyroid gland volumes in a substantial number of children with DS within a narrow age range.

**Subjects and methods**

In this follow-up study in children with Down syndrome, thyroid hormone parameters including anti-TPO antibodies were analyzed at a mean age of 10.7 years, 8.7 years after the end of a single-center RCT in which the effects of thyroxine vs placebo administration between the neonatal period and the age of two years were compared (4, 5). The initial study was conducted between June 1999 and October 2003. Down syndrome neonates were randomized to receive either thyroxine or placebo, and treatment was continued until the age of 24 months. Thyroxine doses were adjusted to reach and maintain normal plasma TSH (0.4–4.0U/L) and high-normal plasma-free T4 (FT4) concentrations (18–24 pmol/L). The daily thyroxine dose decreased from 8 µg/kg at randomization to a little more than 4 µg/kg at age 9 months.

The institutional Medical Ethics Committee approved both the initial and follow-up study. All children who completed the original trial were eligible for inclusion in the follow-up study (Fig. 1). Results on psychomotor development and growth were published previously (5). The purpose of this part of the study was to analyze the effect of thyroxine vs placebo on the thyroid hormone parameters 8.7 years after the end of the trial. Plasma TSH and free thyroxine (FT4) concentration were measured in venous blood two months and 8.7 years after cessation of the trial medication. Anti-TPO antibodies were measured at ages 12 months, 24 months and 10.7 years. Ultrasound of the thyroid gland, to examine the presence of signs of thyroiditis, was performed at the age of 10.7 years.

**Measurements**

**Thyroid function parameters**

Plasma TSH was measured with an electrochemiluminescence immunoassay (E170, Roche Diagnostics). The detection limit was 0.01 U/L, and the total assay variation was less than 5%. The TSH reference interval was 0.5–5.0 U/L. FT4 was measured by time-resolved fluoroimmunoassay (PerkinElmer). The detection limit was 2.0 pmol/L, and the total assay variation was...
5–8%. The FT4 reference interval was 10.0–23.0 pmol/L. Anti-TPO was measured by chemiluminescence immunoassay (LUMI-test anti-TPO, Thermo Fisher Scientific). The detection limit was 30 kU/L and the total assay variation was 8–12%. An anti-TPO concentration of \( \geq 60 \) kU/L was rated as a positive test result.

**Ultrasonography**

Two experienced pediatric radiologists who were blind to the treatment mode during the trial, performed the thyroid ultrasonography using a Philips iU22 in combination with an L17-5 broadband linear array transducer (Philips Medical Systems). Besides thyroid volume (length \( \times \) width \( \times \) depth \( \times 0.52 \) for both thyroid lobes, where the volume of the isthmus was not taken into account), echogenicity, ultrasonographic texture (homogeneous and heterogeneous), irregularities (nodules and cysts) and vascularization were assessed. Autoimmune thyroiditis was defined as an anti-TPO concentration \( \geq 60 \) kU/L or ultrasonographic signs of thyroiditis. Thyroid volume was compared to published reference values for Dutch children of the same age (15).

**Statistical analysis**

Results were compared using chi-square test, Fisher’s exact test, the independent samples \( t \)-test + one-sample \( t \)-test (group statistics, thyroid hormone concentrations and thyroid volume) and binomial test (thyroid volume data compared with Dutch reference data). Statistical analyses were carried out with IBM SPSS Statistics 23.0.0.0. \( P \) values <0.05 were considered statistically significant.

**Results**

**Participants**

One hundred and eighty-one children completed the original RCT of whom 123 participated in the follow-up study (Fig. 1). Analyses to trace possible selection bias revealed that in the thyroxine group, the children participating in the follow-up study showed a smaller delay in mental development at 24 months than the total thyroxine group that completed the original RCT (difference: 0.6 months, 95% confidence interval (CI): -1.2 to 0.0, \( P=0.04 \)). The baseline characteristics of the 123 children in the follow-up study are given in Table 1 and were similar in the thyroxine and placebo groups. There were no significant differences in comorbidity, including cardiac, pulmonary or neurological disease (5). During the RCT, daily thyroxine doses were adjusted at regular intervals to maintain plasma TSH in the reference interval and FT4 concentrations in the higher end of the reference interval. In the follow-up study, measurements were not available in all patients, mainly due to lack to cooperate with blood withdrawal. TSH and FT4 concentrations were

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**Figure 1**

Flow diagram representing the original trial and the follow-up study at the age of 10.7 years.
Table 1  Baseline characteristics of the thyroxine and placebo groups in the follow-up study, given for the total follow-up group (n = 123) and the subgroup without evidence of autoimmune thyroiditis, currently not treated with thyroxine (n = 71).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total follow-up group</th>
<th>Subgroup without treatment/autoimmunity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thyroxine group, n = 64</td>
<td>Placebo group, n = 59</td>
</tr>
<tr>
<td></td>
<td>Thyroxine group, n = 40</td>
<td>Placebo group, n = 31</td>
</tr>
<tr>
<td>Mean age at follow-up, years (s.d.)</td>
<td>10.7 (0.04)</td>
<td>10.7 (0.08)</td>
</tr>
<tr>
<td></td>
<td>10.7 (0.04)</td>
<td>10.7 (0.07)</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>33/31</td>
<td>31/28</td>
</tr>
<tr>
<td></td>
<td>17/23</td>
<td>14/17</td>
</tr>
<tr>
<td>Gestational age at birth, weeks (s.d.)</td>
<td>38.3 (1.4)</td>
<td>38.6 (1.3)</td>
</tr>
<tr>
<td></td>
<td>38.3 (1.4)</td>
<td>38.5 (1.3)</td>
</tr>
<tr>
<td>Mean birth weight, grams (s.d.)</td>
<td>3058 (535)</td>
<td>3073 (531)</td>
</tr>
<tr>
<td></td>
<td>3127 (559)</td>
<td>3085 (490)</td>
</tr>
<tr>
<td>Age at trial entry, days (s.d.)</td>
<td>24.3 (3.3)</td>
<td>24.2 (3.3)</td>
</tr>
<tr>
<td></td>
<td>24.1 (2.7)</td>
<td>23.5 (3.4)</td>
</tr>
<tr>
<td>TSH at trial entry, U/L (s.d.)</td>
<td>6.8 (3.3)</td>
<td>7.1 (3.7)</td>
</tr>
<tr>
<td></td>
<td>6.5 (2.7)</td>
<td>6.2 (2.6)</td>
</tr>
<tr>
<td>FT4, at trial entry, pmol/L (s.d.)</td>
<td>18.6 (3.4)</td>
<td>18.5 (2.8)</td>
</tr>
<tr>
<td></td>
<td>18.5 (2.7)</td>
<td>18.7 (2.4)</td>
</tr>
<tr>
<td>Hypothyroidism at follow-up, n (%)</td>
<td>7 (11.1)</td>
<td>10 (17.2)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hyperthyroidism at follow-up, n (%)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Signs of puberty, male/female</td>
<td>15/9</td>
<td>15/8</td>
</tr>
<tr>
<td></td>
<td>9/7</td>
<td>5/5</td>
</tr>
<tr>
<td>Mental age equivalent, mean (s.d.)</td>
<td>51.6 (10.7)</td>
<td>50.9 (13.6)</td>
</tr>
<tr>
<td></td>
<td>53.6 (9.7)</td>
<td>52.3 (11.9)</td>
</tr>
</tbody>
</table>

available in 113 children (91%; 59 in the thyroxine group (29 males/30 females) and 54 children in the placebo group (28 males/26 females)).

Thyroid hormone parameters

TSH and FT4 concentrations were analyzed in the subgroup of children currently not on thyroid medication and without evidence of autoimmune thyroiditis. This group consisted of 71 children; 40 in the thyroxine group (17 males/23 females) and 31 in the placebo group (14 males/17 females) (Fig. 1).

At original trial entry, no significant differences were seen in either TSH or FT4. As expected, during the trial, at ages 6, 12 and 24 months, FT4 were significantly higher and TSH concentrations significantly lower in the thyroxine-treated group compared with that in the placebo group (Table 2). Two months after cessation of trial medication, at age 26 months, TSH was significantly higher in the thyroxine-treated group (6.9 U/L) compared with that in the placebo group (5.0 U/L) (P = 0.002), whereas FT4 concentrations did not differ (P = 0.40). At the follow-up age of 10.7 years, TSH concentrations did not differ (P = 0.83), but FT4 was significantly higher in the thyroxine-treated group compared with that in the placebo group (14.1 and 13.0 pmol/L respectively, P = 0.02) (Table 2, Fig. 2). Log-linear regression analysis of TSH and FT4 concentrations was not possible due to the small sample size.

TSH and FT4 concentrations in the subgroups of thyroxine- and placebo-treated children (N = 40 and 31 respectively) were also compared with TSH and FT4 in the total groups of thyroxine- and placebo-treated children during the trial (N = 99 and 97 respectively; ages 6, 12 and 24 months), and no statistically significant differences were found (differences in FT4 between subgroup of 40 thyroxine-treated patients compared with total group of 99 thyroxine-treated patients in original trial: trial age 6 months: mean difference: -0.12 pmol/L (95% CI: -1.71 to 1.47), P = 0.88; trial age 12 months: mean difference:

Table 2  Plasma TSH and FT4 concentrations in the subgroup of patients without evidence of autoimmune thyroiditis, currently not treated with thyroxine. Data are given in mean (s.d.), TSH in U/L, FT4 in pmol/L (to convert FT4 to ng/dL divide by 12.87).

<table>
<thead>
<tr>
<th>Trial entry age 0.8 months</th>
<th>Thyroxine group n = 40</th>
<th>Placebo group n = 31</th>
<th>Mean difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>7.1 (3.7)</td>
<td>6.2 (2.6)</td>
<td>0.9</td>
<td>0.28</td>
</tr>
<tr>
<td>FT4</td>
<td>18.5 (2.7)</td>
<td>18.7 (2.4)</td>
<td>-0.2</td>
<td>0.78</td>
</tr>
<tr>
<td>During trial age 6 months</td>
<td>TSH</td>
<td>1.3 (1.5)</td>
<td>5.2 (2.7)</td>
<td>-3.9</td>
</tr>
<tr>
<td></td>
<td>FT4</td>
<td>21.9 (5.0)</td>
<td>14.5 (1.9)</td>
<td>7.4</td>
</tr>
<tr>
<td>During trial age 12 months</td>
<td>TSH</td>
<td>1.4 (1.4)</td>
<td>4.9 (2.5)</td>
<td>-3.5</td>
</tr>
<tr>
<td></td>
<td>FT4</td>
<td>20.8 (4.7)</td>
<td>14.1 (2.1)</td>
<td>6.7</td>
</tr>
<tr>
<td>During trial age 24 months</td>
<td>TSH</td>
<td>1.6 (1.8)</td>
<td>5.2 (1.9)</td>
<td>-3.6</td>
</tr>
<tr>
<td></td>
<td>FT4</td>
<td>20.4 (3.8)</td>
<td>13.9 (1.8)</td>
<td>6.5</td>
</tr>
<tr>
<td>After trial age 26 months</td>
<td>TSH</td>
<td>6.9 (3.3)</td>
<td>5.0 (1.8)</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>FT4</td>
<td>14.5 (3.0)</td>
<td>14.0 (1.9)</td>
<td>0.5</td>
</tr>
<tr>
<td>After trial age 10.7 years</td>
<td>TSH</td>
<td>3.8 (2.5)</td>
<td>3.9 (1.8)</td>
<td>-0.1</td>
</tr>
<tr>
<td></td>
<td>FT4</td>
<td>14.1 (2.6)</td>
<td>13.0 (1.6)</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Thyroxine treatment in Down syndrome

N Zwaveling-Soonawala and others

Clinical Study

0.043 pmol/L (95% CI: −1.47 to 1.55), P=0.96; trial age 24 months: mean difference: −0.25 pmol/L (95% CI: −1.48 to 0.98), P=0.68; differences in FT4 between subgroup of 31 placebo treated patients compared with the total group of 97 placebo treated patients in the original trial: trial age 6 months: mean difference: −0.07 pmol/L (95% CI: −0.77 to 0.63), P=0.84; trial age 12 months: mean difference: −0.25 pmol/L (95% CI: −1.03 to 0.54), P=0.53; trial age 24 months: mean difference: −0.18 pmol/L (95% CI: −0.86 to 0.49), P=0.58).

Thyroid autoimmunity

Anti-TPO was measured in 104 children. The proportion of children with positive anti-TPO at the age of 12 months, 24 months and 10.7 years were 1, 6 and 25% respectively.

Thyroid autoimmunity

Anti-TPO was measured in 104 children. The proportion of children with positive anti-TPO at the age of 12 months, 24 months and 10.7 years were 1, 6 and 25% respectively.

Ultrasound of the thyroid gland was performed in 120 children. Clear signs of thyroiditis were present in seven children (four in the thyroxine group and three in the placebo group). Except for one child, all these children were positive for anti-TPO.

Based on these findings, 8.7 years after the end of the RCT, 26 children could be classified as having thyroid autoimmunity. This included ten out of 54 (18.5%) in the thyroxine group and 16 out of 50 (32%) in the placebo group (Fig. 3). Ten of them were on thyroid medication (4 in the thyroxine group, 6 in the placebo group). Treatment had been started by the patients’ own pediatrician and indications for treatment were overt hypothyroidism in nine children and hyperthyroidism in one child (in the placebo group; block and replace treatment with carbimazole and thyroxine). No difference was found in prevalence of anti-TPO between boys and girls.

Thyroid volume

For the total group of 120 children (61 thyroxine group/59 placebo group, three missing data) thyroid volume assessed by ultrasonography was 3.56 mL (s.d. 1.48) in the thyroxine group and a similar 3.64 mL (s.d. 1.63) in the placebo group (P=0.77). Also for the subgroup of children without evidence of autoimmune thyroiditis, thyroid volume was similar; thyroxine group (n=38) 3.46 mL (s.d. 1.14) and placebo group (n=31) 3.42 mL (s.d. 1.29).


When comparing thyroid volume of the subgroup without evidence of autoimmune thyroiditis (n=69) to the Dutch reference range at age 11 years (median: 5.5, range: 3–9 mL), the mean volume of 3.4 mL (s.d. 1.2; range: 0.5–7.5 mL) was significantly lower (P<0.01) with a thyroid volume below 3 mL in 38 per cent of cases and below 5.5 mL in 97 per cent of cases (P<0.01) (15).

### Discussion

In this follow-up study of an RCT in which children with Down syndrome were treated with thyroxine or placebo between the neonatal period and the age of two years, we found a slightly, but statistically significant higher FT4 concentration (mean difference 1.14 pmol/L, P=0.02) at the age of 10.7 years in the thyroxine group. This slightly higher FT4 concentration in combination with a similar TSH concentration suggests a change in the HPT axis set point induced by the thyroxine treatment during the first two years of life. In addition, there was an increasing incidence of thyroid autoimmunity, measured by the presence of anti-TPO, from 1% at age 12 months to 6% at age 24 months and 25% at age 10.7 years, and there was a trend toward a greater percentage of children with anti-TPO positivity in the placebo group (18.5%) although not statistically significant (P=0.12 at age 10.7 years). Finally, thyroid volume at age 10.7 years (mean: 3.4 mL; range: 0.5–7.5 mL) was significantly lower (P<0.01) compared with reference values (5.5 mL; range: 3–9 mL) and was similar in the thyroxine and placebo group.

In healthy individuals, plasma FT4 and TSH concentrations show inter-individual differences leading to wide reference intervals. The intra-individual variability in plasma FT4 and TSH, however, is much smaller, suggesting that each individual has his or her own specific hypothalamus–pituitary–thyroid (HPT) axis set point that seems to remain stable (16, 17).

The HPT axis set point develops during the fetal period followed by slowly decreasing TSH and minimal change in FT4 concentrations in the first 2 years of life (6, 7, 8). During the fetal period, the HPT axis seems susceptible to permanent changes driven by environmental factors. An example of the fetal environment influencing the postnatal (F)T4 set point is the biochemical phenotype of infants born to inadequately treated mothers with Graves’ disease. In these infants, Kempers et al. described normal TSH concentrations accompanied by low FT4 concentrations (18). Another example is severe primary congenital hypothyroidism (CH), e.g. thyroid agenesis. When treating patients with this condition with thyroxine, achieving a normal TSH concentration is accompanied by FT4 concentrations above the age-specific reference range interval, suggesting an altered HPT axis set point possibly caused by a hypothyroid intra-uterine environment (19, 20). In this study, early intervention with thyroxine treatment after the fetal period seems to have led to an altered TSH-FT4 set point. During the trial, TSH concentrations were significantly lower and FT4 significantly higher (~7 pmol/L) in the thyroxine treatment group (P<0.001) (Table 2). Two months after treatment, TSH was significantly higher (P=0.002) in the thyroxine-treated group, probably due to a recovery of the HPT axis after a long period of thyroxine supplementation.

In general, the relationship between TSH and FT4 is presumed to be log-linear (21). Although it has been suggested that the TSH-FT4 relationship may be more complex and nonlinear (22). Due to the small sample size, log-linear regression analysis was not possible in our study. Physiologically, transient set point changes occur during fasting and as a result of diurnal variation, whereas a permanent set point shift takes place with aging characterized by relatively higher TSH concentrations. Certain non-physiological circumstances may also cause short-term alterations in TSH/FT4 set point, including the effects of acute inflammation on the HPT axis leading to decreased FT4 levels accompanied by normal TSH levels (23, 24, 25). These changes, however, are temporary and do not persist when circumstances are reversed. In our study, the set point change is observed many years after the discontinuation of thyroxine treatment. The mechanism responsible for this persistent set point change may lie in epigenetic modifications of genes involved in TSH/FT4 set point development induced by the thyroxine treatment. In a twin study of neonatal screening T4 results, we were able to emphasize the importance of the fetal environment in neonatal T4 set point determination and hypothesized that the mechanism underlying T4 set point determination lies in epigenetic modifications during the fetal period influencing the maturing HPT axis (10). Perhaps thyroxine treatment during the first
two years of life, in a period in which the HPT axis is not yet completely mature, may also lead to epigenetic modifications inducing a set point change that persists later on in life.

We do acknowledge that in both groups FT4 concentrations were within the normal range and that the observed difference in FT4 between the thyroxine and placebo treated groups is small, based on a single measurement, and probably, without clinical consequences. However, the FT4 difference did reach statistical significance and may point to an interesting new mechanism influencing the maturing HPT axis set point.

At age 10.7 years, 25% of the children with Down syndrome in this study were anti-TPO positive. This is in line with previous findings in children with Down syndrome and emphasizes the much higher prevalence of thyroid autoimmunity in Down syndrome compared with non-Down syndrome children (11, 12). In comparison, in a study of 440 healthy school children with a similar mean age to our study group, anti-TPO antibodies were found in only 3.2% for boys and 5.8% for girls (26). In our study, anti-TPO positivity increased with age with a prevalence of 1% at age 12 months, 6% at age 24 months and 25% at age 10.7 years. In a ten-year longitudinal study of thyroid function in children with Down’s syndrome, a similar increase in anti-thyroglobulin antibodies (3% at age 1 year, 25% at age 10 years) and anti-TPO (5% at age 1 year, 37% at age 10 years) was observed (27). Combined, this suggests an increase in anti-TPO positivity of approximately 2.2–3.6% per year. Although not statistically significant ($P=0.12$), there was a trend toward a greater percentage of children with thyroid autoimmunity in the placebo group (32%) compared with the thyroxine-treated group (18.5%) in our study and may indicate an effect of thyroxine therapy on the development of autoimmune thyroiditis. A possible protective effect of levothyroxine treatment in anti-TPO positive (non-Down syndrome) patients has been described in previous studies (13, 14). The mechanism might be decreased presentation of thyroidal autoantigens due to decreased TSH levels during levothyroxine treatment.

Thyroid volume was similar in the thyroxine-treated and placebo-treated groups, also for the subgroup of children without evidence of autoimmune thyroiditis. However, the observed thyroid volume of approximately 3.4 mL was significantly lower than the reference value of 5.5 mL (range: 3–9 mL) for Dutch children at age 11 years ($P<0.01$) (15). We found several reports on thyroid ultrasonography findings in autoimmune thyroiditis in Down syndrome, but hardly any studies on thyroid volume in the absence of autoimmunity. Cebecci et al. reported a high percentage of thyroid hypoplasia in children with Down syndrome, but these children were all on thyroid medication because of congenital or acquired hypothyroidism (28). Lughetti et al. reported on thyroid ultrasonography results at age 10 years in Down syndrome (90% was classified as normal and 10% as hypoplasia) but did not mention the actual measurements (27). Our finding of overall reduced thyroid volume in children with Down syndrome also in the subgroup without thyroid autoimmunity supports the hypothesis that the observed TSH elevation in Down syndrome may be due to a subtle form of congenital hypothyroidism associated with mild thyroid hypoplasia. Very recently thyroid dysgenesis was described in a Down syndrome murine model (transgenic Dyrk1A mice) although thyroid volume in these mice was increased (29).

A limitation of this study is that we measured FT4 and TSH at only one point of time later in life. Another limitation is that results from children with Down syndrome cannot be simply extrapolated to the general population. A third limitation is that there may be a selection bias as the thyroxine treated children participating in the follow-up study had a smaller delay in the development than the total thyroxine group from the original RCT. Further analysis showed that this could not be explained by difference in FT4 concentrations during the T4 treatment in the trial. Important strengths of this study are that the study groups were initially enrolled in an RCT resulting in groups with similar baseline characteristics and that all children were studied within a very small age range. Other strengths are the longitudinal character and the long period of follow-up.

In conclusion, in this study in children with Down syndrome, thyroxine treatment during the first two years of life seems to have resulted in a small change in the FT4 set point measurable almost 9 years later. We hypothesize that thyroxine treatment, in the period that the HPT is not yet completely mature, may have induced epigenetic modifications leading to an FT4 set point change that persists later on in life. Furthermore, there was a trend toward less development of thyroid autoimmunity in the thyroxine treatment group vs the placebo group, suggesting a protective effect of the early thyroxine treatment. Lastly, thyroid volume was lower compared to reference values suggesting Down-specific thyroid hypoplasia.
Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

The authors wish to thank Professor Raoul Hennekam for carefully reading the manuscript.

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Received 16 October 2016
Revised version received 25 January 2017
Accepted 30 January 2017