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Low FT4 Concentrations around the Start of Recombinant Human Growth Hormone Treatment: Predictor of Congenital Structural Hypothalamic-Pituitary Abnormalities?

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Central hypothyroidism · Nonacquired growth hormone deficiency · Growth hormone treatment · Hypothalamic-pituitary abnormalities · Neonatal screening

Abstract
Background: Growth hormone (GH) treatment may unmask central hypothyroidism (CeH). This was first observed in children with GH deficiency (GHD), later also in adults with GHD due to *acquired* "organic" pituitary disease. We hypothesized that newly diagnosed CeH in children after starting GH treatment for nonacquired, apparent isolated GHD points to *congenital* "organic" pituitary disease. ***Methods:*** Nationwide, retrospective cohort study including all children with nonacquired GHD between 2001 and 2011 in The Netherlands. The prevalence of CeH, hypothalamic-pituitary (HP) abnormalities, and neonatal congenital hypothyroidism screening results were evaluated. ***Results:*** Twenty-three (6.3%) of 367 children with apparent isolated GHD were prescribed LT4 for presumed CeH within 2 years after starting GH treatment. Similarly to children already diagnosed with multiple pituitary hormone deficiency, 75% of these 23 had structural HP abnormalities. In children not prescribed LT4, low pre- or post-GH treatment FT4 concentrations were also associated with structural HP abnormalities. Neonatal screening results of only 4 of the 23 children could be retrieved. ***Conclusion:*** In children with nonacquired, apparent isolated GHD, a diagnosis of CeH after, or a low FT4 concentration around the start of GH treatment, is associated with congenital structural HP abnormalities, i.e., "organic" pituitary disease. Neonatal values could not be judged reliably.

Introduction

Since its introduction in the mid-1980s, treatment with human recombinant growth hormone (GH) has become common practice in the fields of pediatric and adult endocrinology [1–3]. The influence of GH administration on the hypothalamic-pituitary-thyroid axis is well

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recognized [4]. In *non*-GH-deficient individuals, GH administration may result in slightly lower plasma or serum-free thyroxine (FT4) concentrations in combination with higher triiodothyronine (T3) concentrations and almost unchanged thyroid-stimulating hormone (TSH) concentrations [5–7]. These changes in thyroid hormone levels are usually temporary and are thought to be caused by increased T4 to T3 conversion and inhibition of TSH secretion [5]. However, often FT4, T3, and TSH do not show any changes [8, 9].

In contrast to non-GH-deficient individuals, in patients *with* GH deficiency (GHD) changes are often more pronounced and in the last few decades, it has become clear that GH treatment in patients with GHD may unmask central hypothyroidism (CeH). CeH is defined as an FT4 concentration below the age-specific reference interval, in combination with a low, normal or slightly elevated TSH concentration [10]. Unmasking refers to a decrease in FT4 from within the reference range, to below the reference range. This was first observed in children with an initial diagnosis of isolated GHD, who were subsequently reclassified as having multiple (or combined) pituitary hormone deficiency (MPHD) [11]. Later on, it was also reported in adults, especially in those with acquired GHD due to "organic" pituitary disease, i.e., trauma, tumor or after pituitary surgery [12–14].

In the past 15 years, we have also encountered several children with CeH shortly after starting GH treatment for apparent isolated GHD. Additional testing revealed central adrenal insufficiency in a number of these patients. All patients had structural hypothalamic-pituitary (HP) abnormalities mainly consisting of an ectopic posterior pituitary and/or pituitary hypoplasia. In a recent MRI study in early childhood GHD, it was reported that all children with GHD as part of MPHD had complex pituitary defects (i.e., ectopic posterior pituitary, with or without pituitary hypoplasia, and pituitary stalk or midline abnormalities). In contrast, most of the children with isolated GHD had a normal pituitary anatomy or only isolated pituitary hypoplasia [15]. Nowadays, most pediatric endocrinologists perform additional pituitary function tests and MRI of the HP region in children diagnosed with GHD. Since MRI is usually not possible in young children without the use of general anesthesia, imaging is often postponed until an older age, especially when other pituitary deficiencies seem to be absent. Since the gonadal axis cannot be reliably tested beyond the first months of life, and the adrenal axis requires dynamic testing, the decision to perform MRI under general anesthesia may be solely guided by the presence of CeH. This raises the question whether low FT4 concentrations and/or a diagnosis of CeH at initiation of GH treatment for apparent GHD indicates congenital pituitary malformation.

In The Netherlands, each year 8–10 children with CeH are detected in the neonatal screening program for congenital hypothyroidism (CH; Dutch incidence: approximately 1 in 16,000) [16]. Approximately 75% of these children have congenital MPHD, usually including GHD and most of these children have pituitary malformations [16, 17]. These pituitary malformations are similar to those seen in children initially presenting with isolated GHD which turns out to be part of MPHD including CeH. These children apparently had not been detected in the Dutch neonatal screening program for CH. We hypothesized that their neonatal thyroid hormone concentrations may be at the lower limit of the reference range but not low enough to be detected by neonatal screening.

To study the occurrence of CeH after initiation of GH treatment in children with congenital GHD, we conducted a nationwide, retrospective cohort study including all children with congenital GHD diagnosed between January 2001 and January 2011 in The Netherlands. To identify MPHD, we studied the presence of structural and/or functional HP abnormalities. In addition, we attempted to retrieve neonatal CH screening results.

Subjects and Methods

Patients

All children diagnosed with congenital GHD between January 2001 and January 2011 in the Netherlands, and younger than 18 years at the initiation of GH treatment, were eligible for the study. Patients were retrieved from the database of the Dutch Growth Research Foundation (DGRF). GHD was defined as a maximal plasma GH concentration ≤20 mIU/L in two GH stimulation tests combined with a sex- and age-specific serum IGF-1 concentration <0 SD, or a maximal GH ≤30 mIU/L in combination with an IGF-1 <–2 SD (most Dutch laboratories report GH concentrations in milli-international units/liter; to convert to microgram/liter, divided by 3) [18]. Exclusion criteria were acquired GHD (brain tumor, cranial or total body irradiation, or traumatic brain injury), possible (partial) GH resistance, and GH treatment for indications other than GHD. Children treated with levothyroxine (LT4), hydrocortisone, sex steroids or desmopressin *prior to* the start of GH treatment were classified as having MPHD (two or more pituitary deficiencies). The occurrence of CeH after the initiation of GH treatment was defined as a low plasma FT4 concentration in the presence of an inappropriately low, normal or mildly elevated TSH, for which LT4 treatment had been started by the child's doctor [19]. In The Netherlands, the lower limit of the adult reference interval of most FT4 assays is between 8 and 12 pmol/L, with an average of around 10 pmol/L; the average upper limit is 23 pmol/L (personal communications). The lower limit of most TSH assays

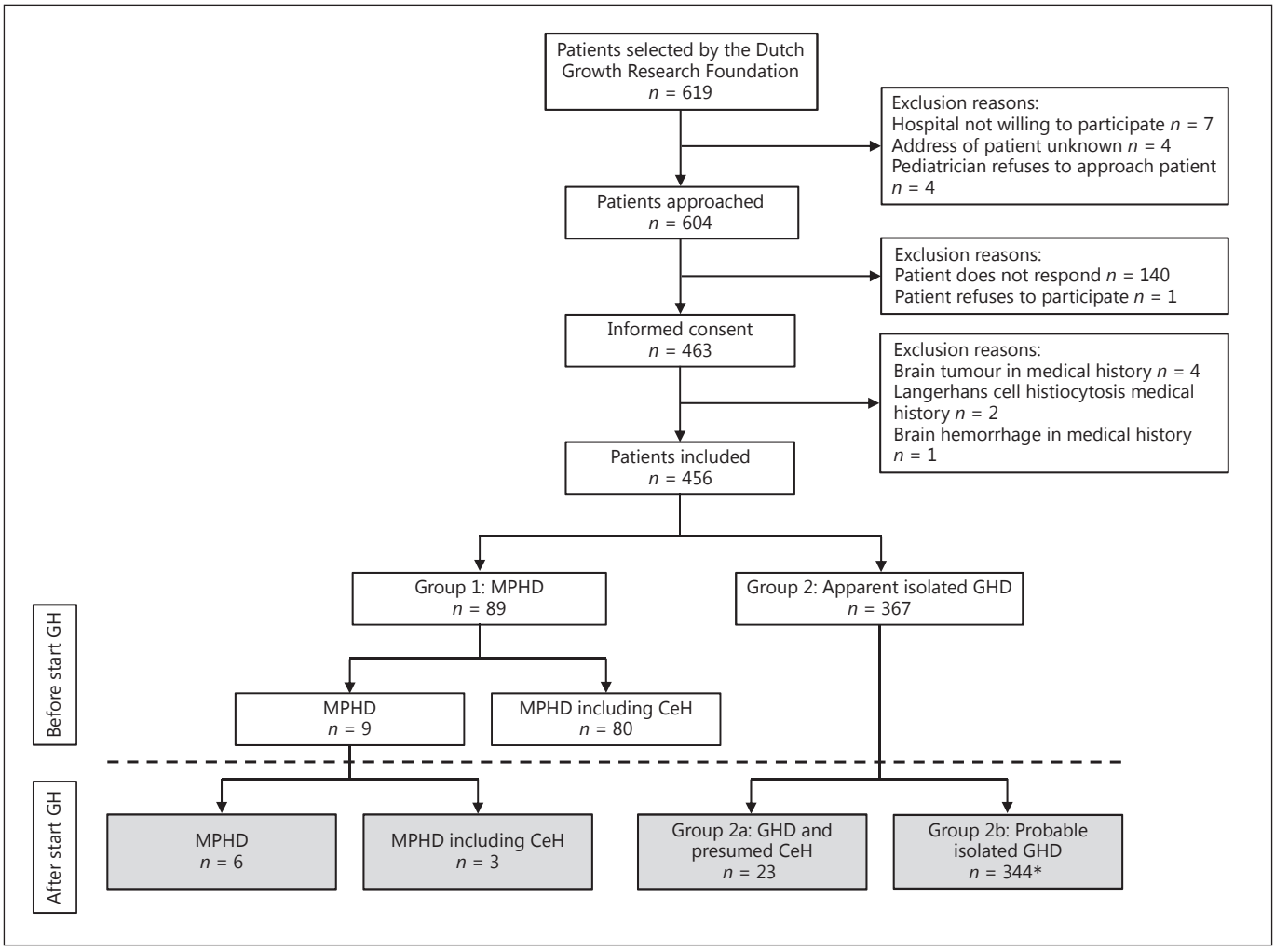

Fig. 1. Selection of patients. White boxes represent the classification of patients at the start of growth hormone treatment; gray boxes represent the classification of patients 2 years after the start of growth hormone treatment. CeH, central hypothyroidism; GHD, growth hormone deficiency; MPHD, multiple pituitary hormone deficiency. * Two patients with probable isolated GHD were prescribed thyroxine for potential primary hypothyroidism after the start of GH treatment (pretreatment FT4 and TSH concentrations: 15.0 pmol/L and 5.6 mIU/L, and 14.1 pmol/L and 9.83 mIU/L, respectively).

is between 0.3 and 0.5 mIU/L, with an average of around 0.4 mIU/L; the highest reported upper limit is 5.0 mIU/L. Since innocent and transitory changes in thyroid hormone levels after the initiation of GH treatment usually resolve within 1–2 years, we set the time window for the diagnosis of CeH to be made at 2 years after the start of GH treatment [20].

Data were extracted from the children's hospital records and DGRF database files, and were cross-checked with data stored by the National Institute for Public Health and the Environment (RIVM-DVP, the organization responsible for the Dutch neonatal screening). MRI reports were retrospectively retrieved and classified as either "normal" or "abnormal" (reported as ectopic posterior pituitary; absent or thin pituitary stalk; pituitary hypoplasia; empty sella). In addition, parents were asked to participate in a structured telephonic interview to collect information on clinical factors influencing neonatal thyroid function. To keep the investigators blinded, with a few exceptions, interviews were performed before retrieval of data from hospital records, database files, and neonatal screening results. According to the Dutch neonatal screening definitions, children were classified as "premature" if gestational age was <36 weeks in combination with a birth weight <2.5 kg. "Non-thyroidal illness" was suspected when the interview revealed birth asphyxia (5 min Apgar score <7), use of intravenous antibiotics (for probable perinatal infection), weight loss of 10% or more (often resulting from feeding problems) or gastrointestinal surgery preceding the neonatal screening.

The Medical Ethics Committee of the Academic Medical Center, the Dutch national GH advisory board and the RIVM approved the study. Written informed consent was obtained from all children and their parents.

Table 1. Baseline characteristics of the 456 patients at the start of GH treatment

Characteristics	Group 1 (n = 89); GHD probably within the framework of congenital MPHD[a]	Group 2 (n = 367); apparent isolated GHD	
		group 2a (n = 23); GHD and presumed CeH	group 2b (n = 344[b]); probable isolated GHD
Gender (male), n (%)	63 (70.8)	15 (65.2)	230 (66.9)
Gestational age, weeks	39.0 (26.1 to 42.7)	40.0 (30.0 to 42.0)	40.0 (25.0 to 43.0)
Birth weight (SD for GA)	–0.54 (–3.68 to 1.69)	–0.30 (–2.70 to 2.45)	–0.37 (–4.15 to 3.52)
Age, years	3.90 (0.17 to 16.11)[c]	4.36 (1.89 to 14.34)	5.62 (0.54 to 15.68)
Height SDS	–3.00 (–6.89 to –0.03)	–2.89 (–4.48 to –1.72)	–2.92 (–6.69 to –0.72)
Bone age delay, years	–1.3 (–6.6 to 1,0)	–1.4 (–4.0 to 0.7)	–1.2 (–11.4 to 0.6)
Maximum stimulated GH concentration, mIU/L[d]	8.6 (0.1 to 34.2)	13.0 (3.2 to 29.9)	16.1 (1.0-61.6)[e]
Thyroid function parameters[f]	Not applicable	Before the start of GH treatment	Before the start of GH treatment
FT4, pmol/L		12.3 (7.6 to 19.4)[g,h]	15.1 (7.8 to 24.0)
TSH, mIU/L		2.10 (0.17 to 7.70)	2.34 (0.32 to 6.70)
Thyroid function parameters		After the start of GH treatment	6 months (range 4–8) after the start of GH treatment
FT4, pmol/L		9.9 (6.6 to 12.0)[h]	14.0 (8.2–25.0)
TSH, mIU/L		1.98 (0.12 to 3.30)	2.60 (0.93–7.00)
Thyroid function parameters		After the start of LT4 treatment	12 months (range 9–13) after the start of GH treatment
FT4, pmol/L		16.1 (12.0 to 20.1)	14.8 (8.1–22.8)
TSH, mIU/L		0.39 (0.01 to 3.30)	2.44 (0.52–7.75)
MRI results, n			
Total available MRI results	75	21	213
Normal MRI result	19	5	150
Abnormal MRI result[i]	56	16	63

Bone age delay, delay with respect to calendar age; GHD, growth hormone deficiency; MPHD, multiple (or combined) pituitary hormone deficiency; CeH, central hypothyroidism; SDS, standard deviation. All values are median (min. to max.), except where indicated otherwise. [a] Of the patients with MPHD, GHD was combined with central hypothyroidism only (CeH) in n = 40, with central adrenal insufficiency only (CeA) in n = 4, with central (or hypogonadotropic) hypogonadism only (CeHy) in n = 2, or with diabetes insipidus only (CeDI) in n = 2; in the other patients with MPHD, GHD was combined with both CeH and CeA in n = 36, or with both CeH and CeDI in n = 1; 4 patients had dysfunction of >3 pituitary axes: CeH+CeA+CeHy in n = 2, and CeH+CeA+CeDI in n = 2. [b] Two patients with probable isolated GHD were prescribed thyroxine for potential primary hypothyroidism after the start of GH treatment (pretreatment FT4 and TSH concentrations: 15.0 pmol/L and 5.6 mIU/L, and 14.1 pmol/L and 9.83 mIU/L, respectively). [c] Group 1 vs. groups 2a and 2b, $p < 0.05$. [d] In 6 patients, maximum stimulated GH concentrations were not available, because the diagnosis GHD was based on clinical symptoms (n = 2), or GH was only measured during hypoglycemia (n = 2) or randomly in neonates (n = 2). [e] Group 2b vs. groups 1 and 2a, $p < 0.05$. [f] The lower limits of most FT4 assays are between 8 and 12 pmol/L, with an average around 10 pmol/L. The average upper limit is 23 pmol/L The lower limits of most TSH assays are between 0.3 and 0.5 mIU/L, with an average around 0.4 mIU/L. The highest used upper limit is 5.0 mIU/L. [g] Group 2a vs. group 2b, $p < 0.001$. [h] Difference in FT4 concentration before and after the start of GH treatment within group 2a, $p < 0.001$. [i] Abnormal MRI refers to one or more of the following congenital abnormalities: ectopic posterior pituitary; absent or thin pituitary stalk; pituitary hypoplasia; empty sella.

Neonatal Screening Results

The Dutch neonatal CH screening is primarily (total) T4 based. TSH is measured in the 20% lowest T4 concentrations with an additional T4-binding globulin (TBG) measurement in the 5% lowest T4 concentrations [21]. All screening laboratories use the same T4, TSH, and TBG assays. Quarterly nationwide quality controls guarantee high reproducibility and comparability. Since screening results were stored in a single national RIVM database from 2002 onward, we were not able to retrieve normal results of children born before that year. However, we were able to retrieve abnormal screening results by searching paper archives of regional screening centers.

Statistical Analyses

Patients were divided into two groups: group 1 consisting of patients with MPHD and group 2 consisting of patients with apparent isolated GHD (i.e., no treatment for other pituitary deficiencies at the start of GH treatment). Group 2 was further divided into patients who were prescribed LT4 for presumed CeH within 2 years after starting GH treatment (group 2a), and those who were not (group 2b).

Data are presented as median (min. to max.). Nonnormally distributed data in more than two categories were tested for significance by the Kruskall-Wallis test for nonparametric measurements. Two categories were compared with the Mann-Whitney U test. Categorical data were compared using the χ^2 test. The paired *t* test was used to compare differences within groups during treatment in normally distributed data and the Wilcoxon signed rank test in nonnormally distributed data. A value of $p < 0.05$ was considered statistically significant. Data were analyzed using SPSS version 22 for Windows (IBM SPSS System Inc., Chicago, IL, USA).

Results

Patients

In the DGRF database, inclusion criteria were met by 619 children of whom 604 could be contacted by their participating pediatricians (endocrinologists). Four hundred and sixty-three children and parents agreed to participate, but 7 children were excluded due to previously unnoticed *acquired* GHD, resulting in 456 children (Fig. 1).

Baseline Characteristics, and Thyroid Function before and after Starting GH Treatment

Eighty-nine of the 456 included patients were diagnosed with MPHD (group 1), and 367 with apparent isolated GHD (group 2) before GH treatment. Twenty-three children in group 2 (6.3%) were prescribed LT4 treatment for presumed CeH diagnosed within 2 years after the initiation of GH treatment (after a median of 0.6 years, range 0.1–1.9; group 2a). Three hundred and forty-two of the remaining 344 children (group 2b) were judged as having a normal thyroid function, while 2 children were prescribed LT4 for presumed mild primary hypothyroidism. In 80 of the 89 children in group 1 – the MPHD group –, LT4 treatment was already started before GH treatment, in 3 other children LT4 was started after initiation of GH treatment. Table 1 shows the baseline characteristics of the children in groups 1, 2a, and 2b. Compared to groups 2a and 2b, the children in group 1 were significantly younger at the start of GH treatment (3.90 vs. 4.36 and 5.62 years, respectively). Maximum stimulated GH concentrations were significantly lower in groups 1 and 2a compared to group 2b (8.6 vs. 13.0 and 16.1 mIU/L, respectively).

Before starting GH treatment, the median plasma FT4 concentration of the children in group 2a was significantly lower than in group 2b (12.3 vs. 15.1 pmol/L, respectively; $p < 0.001$), while the median TSH concentrations were similar (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000486033). After starting GH treatment, the median plasma FT4 concentration in group 2a decreased to 9.9 pmol/L. After the start of LT4 treatment, median FT4 increased and TSH decreased from 1.98 to 0.39 mIU/L. In group 2b, the children with presumed "normal" pituitary function, FT4 decreased from 15.1 pmol/L to 14.0 pmol/L, but within 1 year FT4 spontaneously increased to 14.8 pmol/L. TSH did not change significantly.

Figure 2 shows scatterplots of the FT4 and TSH concentrations of the children in groups 2a and 2b before and after initiation of GH treatment. The third time point in these scatterplots represents the FT4 and TSH concentrations on LT4 treatment in group 2a or after 1 year of GH treatment in group 2b. An intriguing observation is that a number of children in group 2b – i.e., the children judged as having a normal thyroid function – had a low or low normal FT4 concentration before and after initiation of GH treatment, and also later on. Twelve of them were still prescribed LT4 more than 2 years after initiation of GH treatment (after a median of 5.1 years, range

Fig. 2. FT4 and TSH concentrations before (**a**, **b**) and after the start of growth hormone treatment (**c–f**), in the children who were (**a**, **c**, **e**) and were not (**b**, **d**, **f**) treated with levothyroxine. The circles in **a**, **c**, and **e** represent the children who were diagnosed with (presumed) central hypothyroidism after the start of growth hormone treatment. **a** Before the start of growth hormone treatment. **c** After the start of growth hormone treatment, but before the start of levothyroxine treatment. **e** After the start of levothyroxine treatment. The circles in **b**, **d**, and **f** represent the children whose thyroid function was judged as "normal" before (**b**) and after (**d**) the start of growth hormone treatment. **f** Their thyroid function approximately 1 year later. The vertical and horizontal lines represent the median FT4 and TSH concentrations, respectively. The vertical and horizontal dashed lines represent the lower and upper limits of the adult FT4 and TSH reference intervals. The lower limits of most FT4 assays are between 8 and 12 pmol/L, with an average around 10 pmol/L. The average upper limit is 23 pmol/L. The lower limits of most TSH assays are between 0.3 and 0.5 mIU/L, with an average around 0.4 mIU/L. The highest used upper limit is 5.0 mIU/L.

(For figure see next page.)

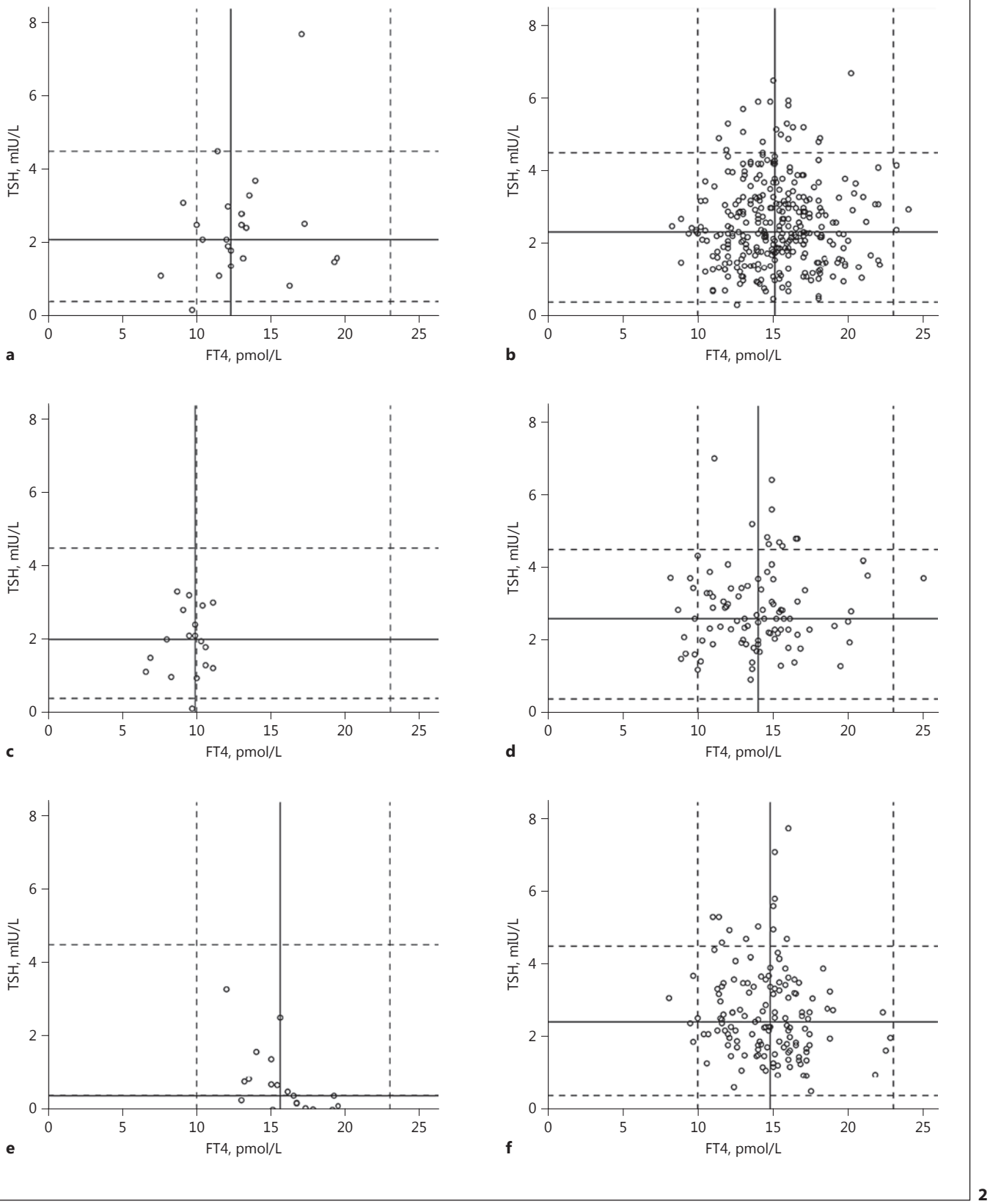

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Table 2. Neonatal screening results of the children with multiple pituitary hormone deficiency versus the children with GH deficiency and newly diagnosed central hypothyroidism, and those with probable isolated GH deficiency at the start of GH treatment

	n	T4 (SD score)	Min. to max.	*n*	TSH, mIU/L	Min. to max.	*n*	TBG, nmol/L	Min. to max.	*n*	T4/TBG ratio	Min. to max.
Neonatal screening results, minus excluded results[a]												
Group 1	26	–2.2	–4.3 to 1.4	20	1.50	0.50 to 4.50	18	195.5	113.0 to 345.0	18	13.0	5.8 to 21.3
Group 2a	4	–1.9	–3.0 to –0.4	3	1.50	1.50 to 2.00	3	160.5	107.5 to 174.0	3	19.6	19.4 to 19.6
Group 2b	126	–0.5[b]	–2.9 to 2.3	34	1.50	0.50 to 5.50	10	166.0	105.0 to 224.0	10	19.8	12.6 to 29.6

	T4 (SD score)	TSH, mIU/L	TBG, nmol/L	T4/TBG ratio	Result
Individual neonatal screening results group 2a					
Subject					
6[c]	–3.8	4.00	71.5	18.2	Negative
7	–0.4				Negative
16[c]	–1.8	1.00	184.0	17.9	Negative
17[d]	–3.0	1.50	107.5	19.6	Positive
19	–1.7	1.50	174.0	19.6	Negative
20	–2.0	2.00	160.5	19.4	Negative

[a] Sixty neonatal screening results were excluded from the analysis because of prematurity ($n = 30$) or probable nonthyroidal illness($n = 30$). Another 9 results were excluded because of missing perinatal data. [b] Group 2b vs. groups 1 and 2a: $p < 0.05$. [c] Excluded neonatal screening results in group 2a: subject 6, for prematurity (gestational age 30 weeks, birth weight 810 g); subject 16, for probable nonthyroidal illness (breech delivery, Apgar score unknown, transfer to university medical center for unexplained low heart rate). [d] Abnormal first neonatal screening result (very low T4 SD score); patient was referred to a pediatrician, but not treated; FT4 concentration could not be retrieved. For all measurements, medians and means were approximately similar, except for the TSH concentration, which is not distributed normally.

2.9–9.8). Based on the assumption that these children may very well have – at that time still undiagnosed – CeH, and that structural abnormalities of the HP region may support this diagnosis, we compared the MRI results of the children in groups 1, 2a, and 2b, and we analyzed HP MRI results of the children in group 2b in relation to FT4 at the three time points.

MRI Results

HP MRI results of 309 of the 456 included patients were available. Sixteen of 21 (76.2%) MRI studies of patients in group 2a showed congenital abnormalities, similar to the percentage of the children in group 1 (abnormalities in 56 of 75 [74.7%]), but clearly higher than the percentage in group 2b (abnormalities in 63 of 213 [29.6%]). Sixteen of the 344 children in group 2b had FT4 concentrations <10 pmol/L before or at least at one measurement after initiation of GH treatment. In 8 of these 16 cases, MRI results were available and showed congenital abnormalities in 6 cases (75%). When this analysis was repeated in children with a lowest FT4 concentration of 10–11 pmol/L, the percentage was 57% (4 of 7). In children with a lowest FT4 concentration of 11–12 pmol/L, this was 25% (5 of 20). In children with a lowest FT4 concentration of 12–13, 13–14 and ≥14 pmol/L, the percentages were 28.6, 26.7, and 26.8%. In 8 of the 12 children who were prescribed LT4 more than 2 years after the initiation of GH treatment, MRI studies were available, with congenital abnormalities in 5 cases (62.5%).

Neonatal Screening Results

Parents of 400 children were interviewed, and neonatal CH screening results of 225 could be retrieved. Sixty screening results were excluded from analysis because of prematurity ($n = 30$) or probable nonthyroidal illness ($n = 30$). Another 9 were excluded because of missing perinatal data. In total, screening results of 156 children were available for analysis. Screening results of only 4 children in group 2a were retrieved. Their T4 concentrations were clearly lower than in group 2b, but comparable to group 1 (median T4 SD scores –1.9 [group 2a, $n = 4$], –0.5 [group 2b, $n = 126$], and –2.2 [group 1, $n = 26$], respectively; $p < 0.05$ for group 2b vs. groups 1 and 2a) (Table 2). TSH concentrations were similar.

Adrenal Axis Testing Results

Assessment of the HP adrenal axis was performed in 11 of the 23 children in group 2a after initiation of GH treatment. In 6 patients, a classic (250 μg) ACTH test was performed, in 2 a low-dose (1 μg) ACTH test, and in 1 a morning cortisol measurement was used to evaluate the HP adrenal axis. In 2 other patients, the mode of testing or results could not be retrieved. However, in these 2 pa-

tients the medical chart mentioned the prescription of cortisol treatment for insufficient ACTH reserve. Six of these 11 patients were subsequently treated with hydrocortisone, 3 after and 3 before initiation of LT4 treatment. Two children were prescribed hydrocortisone use during stress only. In the 8 patients with an abnormal HP adrenal axis test result and available MRI result, 5 were abnormal (ectopic posterior pituitary, in combination with pituitary hypoplasia [$n = 4$] or a thin pituitary stalk in combination with pituitary hypoplasia [$n = 1$]). All 3 patients with a normal ACTH test result had pituitary hypoplasia.

Discussion

In this large Dutch cohort of children treated with GH for apparent isolated GHD, 23 children (6.3%) were prescribed LT4 treatment for presumed CeH within 2 years after initiation of GH treatment. Similar to children already diagnosed with (congenital) MPHD before starting GH treatment, approximately three-quarter of these children had congenital structural HP abnormalities. In retrospect, 3 of these patients had FT4 concentrations just below the reference range interval before the initiation of GH treatment, but were prescribed LT4 only after further decrease of FT4 concentrations. In children with apparent isolated GHD, *not* prescribed LT4 treatment after initiation of GH treatment, a relationship was found between FT4 concentrations and the presence of anatomic pituitary abnormalities. In these children, the *lower* the pre- or post-GH treatment FT4 concentrations were, the *higher* the chance that MRI revealed congenital pituitary malformations. In children with the lowest FT4 concentrations (one or more times <10 pmol/L), the percentage of pituitary abnormalities was even similar to that in children with MPHD (75%). If all children with apparent isolated GHD, *and* a low FT4 (<10 pmol/L), *and* abnormal MRI are considered to have CeH, the percentage of unmasked CeH around the initiation of GH treatment increases to 7.9% (23 + 6 = 29; 29 of 367 = 7.9%). Several children with newly diagnosed CeH were also diagnosed with central adrenal insufficiency, and were started on hydrocortisone treatment or were prescribed hydrocortisone during periods of stress or illness. Unfortunately, neonatal CH screening results were available for only 4 of the 23 children prescribed LT4. These 4 children had lower neonatal screening T4 concentrations, similar to children who were already diagnosed with MPHD before starting GH treatment. This supports our hypothesis that a (too) low FT4 around the initiation of GH treatment in a child with apparent isolated GHD is a very strong predictor of the presence of MPHD resulting from congenital, structural pituitary abnormalities. The finding that the neonatal CH screening results available in the few children with presumed CeH were lower than average suggests that the CeH, although probably mild, may have been present from birth onwards. However, this observation needs to be validated in a larger group of patients.

This is not the first study to demonstrate lower thyroid hormone concentrations after initiation of GH treatment in children with GHD suggestive of CeH [11, 22, 23]. However, in all previous studies the children showing this phenomenon were already diagnosed with acquired organic pituitary disease or congenital MPHD. The same applies to studies in adults, in which GH treatment was found to unmask CeH in patients with known acquired organic pituitary disease [4]. Our study is the first to demonstrate this phenomenon in children with *presumed* isolated GHD; the low FT4 concentrations were the first clue for additional functional and/or structural pituitary abnormalities. As already mentioned in the introduction, Pampanini et al. [15] recently described brain MRI findings in 68 children diagnosed with GHD before the age of 4 years. All 31 children diagnosed with MPHD had complex pituitary defects, while most of the 37 children diagnosed with isolated GHD only showed isolated pituitary hypoplasia or even a normal pituitary gland. Children with MPHD were diagnosed at a younger age than children with isolated GHD. With respect to the presence or absence of structural HP abnormalities, the results of our study are in line with these results. The children in our study diagnosed with MPHD before the initiation of GH treatment, and the children diagnosed with CeH after the initiation of GH treatment, had a higher percentage of "complex defects" than the children diagnosed with probably isolated GHD.

The Dutch neonatal CH screening consists of a three-step "T4+TSH+TBG" approach, enabling calculation of the so-called "T4/TBG ratio" [16]. The 29 children in this study with (probable) CeH and not detected by neonatal screening had a T4 concentration, or a T4/TBG ratio above the screening cutoffs. If these 29 children really had (mild) congenital CeH, this would raise the Dutch prevalence from 1 case per 16,404 to approximately 1 case per 13,211 [16]. Increasing neonatal screening cutoffs would probably enable the detection of these mild cases of CeH but would result in an increase in false-positive test results.

Genetic results were available in 2 of the 23 children treated with LT4 after initiation of GH treatment. One

child had a mutation in *IGSF1*, another in *POU1F1*, both well-known genetic causes of congenital CeH [24–27]. Like the abnormal pituitary morphology in the other children, these genetic findings support the true nature of the CeH in these 2 children.

Major strengths of our study are the large sample size (n = 456), the high percentage of participating children and parents (74.8%), and the fact that we were able to recruit participants from a complete, national cohort of children with GH treatment for apparent isolated GHD over a period of 10 years. However, our study has some limitations. Firstly, diagnosing CeH is not easy. Although a (very) low FT4 in combination with a normal TSH concentration in the absence of nonthyroidal illness is strongly suggestive of this condition, FT4 concentrations around the lower limit of the reference interval are often difficult to interpret [27, 28]. In addition, the decision to start LT4 treatment in the GH-treated children were made by different pediatric endocrinologists and pediatricians, using different FT4 assays and reference intervals. This may have resulted in over- as well as underestimation of the number of CeH cases. Furthermore, the dataset unfortunately was not complete. Not all children with isolated GHD underwent MRI of the pituitary region. This may have led to over- or underestimation of the number of children with abnormal pituitary morphology. Lastly, neonatal CH screening results were retrieved in only 50% of the included children, and one third had to be excluded from further analyses. This clearly affects the validity of the conclusions.

In summary, in this large retrospective cohort study, CeH was diagnosed in at least 6.3% of children with GH treatment for apparent isolated GHD. Approximately 75% of these children had congenital structural pituitary abnormalities. The same percentage of pituitary abnormalities was found in children with a low or low normal FT4 concentration, not yet diagnosed with CeH. These findings suggest that low FT4 concentrations around the initiation of GH treatment in children with congenital GHD are a predictor of the presence of congenital structural pituitary abnormalities and, with that, the diagnosis MPHD. FT4 concentrations around the lower limit of the reference interval before GH treatment may indicate CeH and require close follow-up and, if necessary, additional diagnostic testing. Although we concur that brain imaging should be performed in every child with apparent isolated GHD, this may be postponed until a later age if FT4 concentrations are repeatedly above the lower tertile of the reference interval, and if periodic adrenal axis testing is normal. Obvious exceptions are children suspected of having a space-occupying brain lesion. Although our results suggest that children diagnosed with CeH around the initiation of GH treatment already had (mild) CeH in the neonatal period, this needs further investigation.

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Disclosure Statement

The authors declare no conflict of interest.

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