TSH and FT4 Concentrations in Congenital Central Hypothyroidism and Mild Congenital Thyroidal Hypothyroidism

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Context: In central hypothyroidism (CeH), free thyroxine (FT4) concentrations are low, whereas thyrotropin (TSH) concentrations may be low, normal, or even slightly elevated due to reduced bioactivity. Congenital CeH (CCeH) may be isolated or part of multiple pituitary hormone deficiencies (MPHD).

Objective: We tested our hypotheses that (1) TSH concentrations have a more U-shaped distribution in children with CCeH compared with children with a normally functioning hypothalamic-pituitary-thyroid axis and (2) TSH concentrations in children with CCeH with MPHD are higher compared with children with isolated CCeH. We also studied whether FT4 levels are helpful in distinguishing CCeH from mild congenital hypothyroidism of thyroidal origin (CH-T).

Methods: Dutch neonatal screening TSH and first diagnostic TSH and FT4 were analyzed in all children diagnosed with permanent CCeH between 1995 and 2012. Controls were children with T4-binding globulin deficiency. FT4 concentrations in CCeH were compared with those in CH-T with TSH values in the same range as those of CCeH.

Results: We studied 120 children with CCeH (isolated CCeH, n = 50; MPHD, n = 70) and 350 control subjects. Screening TSH concentrations were not significantly different ($P = 0.055$), but diagnostic TSH values were significantly different between the CCeH group and the control group ($P = 0.037$). TSH was significantly higher in MPHD compared with isolated CCeH ($P = 0.004$). FT4 concentrations were significantly lower in CCeH compared with mild CH-T ($P < 0.0005$).

Conclusion: TSH values in CCeH have a more U-shaped distribution compared with controls with the highest TSH concentrations in MPHD. FT4 levels were significantly lower in CCeH compared with CH-T. (***J Clin Endocrinol Metab*** **103: 1342–1348, 2018)**

Central hypothyroidism (CeH) is characterized by suboptimal thyroid hormone production due to insufficient stimulation by thyrotropin (TSH) of an otherwise normal thyroid gland (1). CeH may be caused by congenital or acquired disorders of the pituitary gland or hypothalamus, resulting in altered TSH production (1).

TSH is a dimeric glycoprotein hormone composed of two subunits: a hormone-specific β-subunit and an α-subunit that is shared with follicle-stimulating hormone, luteinizing hormone, and chorionic gonadotropin. TSH production is regulated by hypothalamic TSH-releasing hormone (TRH). In addition to stimulating transcription of the α- and β-subunit genes, TRH mediates glycosylation and conjugation of TSH-α and -β subunits and TSH secretion. The absence of normal TRH stimulation leads to impaired pituitary TSH glycosylation, resulting in decreased bioactivity of circulating TSH (1–3).

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Abbreviations: ACTH, adrenocorticotropin; CCeH, congenital central hypothyroidism; CeH, central hypothyroidism; CH-T, congenital hypothyroidism of thyroidal origin; FT4, free thyroxine; GH, growth hormone; HPT, hypothalamus-pituitary-thyroid; MPHD, multiple pituitary hormone deficiencies; PSIS, pituitary stalk interruption syndrome; SD, standard deviation; T4, thyroxine; TBG, thyroxine-binding globulin; TRH, thyrotropin-releasing hormone; TSH, thyrotropin.

The biochemical diagnosis CeH is usually made when a low serum free thyroxine (FT4) concentration is accompanied by a "normal" or low TSH concentration. However, due to the presence of TSH with reduced bioactivity, TSH concentrations as measured by immunoassays are not always normal or low but may also be slightly elevated. This sometimes makes distinguishing CeH from mild primary hypothyroidism a challenge.

In previous studies, TSH concentrations in CeH have been reported to range from well below the reference range up to ~11 mIU/L (upper limit of reference range, 4.0) (4–6). Most of the patients in these reports had acquired/adult-onset CeH (*e.g.*, due to pituitary tumors). There are no reports on TSH concentrations in large groups of patients with congenital CeH (CCeH).

Worldwide, most neonatal screening programs for congenital hypothyroidism are TSH based and detect congenital hypothyroidism of thyroidal origin (CH-T) (7). Only a few countries use a screening method in which, in addition to TSH, total T4 or FT4 are measured (T4+TSH method), enabling detection of both CH-T and CCeH. In the Netherlands, a three-step approach is used in which the initial T4 measurement is followed by measurement of TSH and T4-binding globulin (TBG). This approach has proven to be effective in detecting CCeH and CH-T (8, 9). In the last decade, lowering of the TSH cutoff in TSH-based neonatal screening programs has led to an increase in the detection of mild CH-T accompanied by slightly elevated TSH concentrations, which may be difficult to distinguish from CCeH.

CCeH may be due to isolated TSH deficiency or due to altered TSH secretion within the framework of multiple pituitary hormone deficiencies (MPHD). In general, TSH values in genetic forms of isolated CCeH have been reported to range from undetectable to normal (10–17). In MPHD, TSH values may be low in pituitary defects but higher in hypothalamic defects. The most common cause of congenital MPHD is a malformation of the pituitary gland, the so-called "pituitary stalk interruption syndrome" (PSIS) (18). In PSIS the pituitary stalk malformation thwarts hypothalamic TRH stimulation of pituitary thyrotrope cells, resulting in impaired TSH glycosylation and reduced bioactivity. In this condition TSH, as measured by immunoassays, may be elevated (9).

Because TSH levels in CCeH may be low but also may be elevated (especially in MPHD associated with PSIS), we expected the overall range of TSH concentrations in children with CCeH to have a more U-shaped distribution compared with children with a normally functioning hypothalamus-pituitary-thyroid (HPT) axis. Because CCeH within MPHD is most often caused by PSIS and accompanied by TSH with reduced bioactivity, we hypothesized that TSH concentrations in CCeH within MPHD are higher than in isolated CCeH.

The goals of this study were to study whether TSH concentrations in children with CCeH have a more U-shaped distribution compared with the TSH distribution in children with a normally functioning HPT axis and to test the hypothesis that TSH concentrations in children with CCeH within the framework of MPHD are higher compared with children with isolated CCeH. In addition, we studied whether FT4 levels are helpful in distinguishing CCeH from mild CH-T.

Materials and Methods

Dutch neonatal screening program

The Dutch neonatal screening program consists of a three-step T4-TSH-TBG approach. The initial measurement is total T4, which is expressed as standard deviation (SD) of the daily mean. In the lowest 20% (T4 ≤ -0.8 SD) additional TSH measurements are done, and in the lowest 5% (T4 ≤ -1.6 SD) additional TBG measurements are done. This approach enables calculation of the T4/TBG ratio as an estimation of the FT4 concentration. TBG measurement reduces the number of false positives due to harmless TBG deficiency.

Up until 30 June 2012, the T4/TBG ratio in the Dutch screening program was only used for T4 results between -3.0 and -1.6 SD. Children with T4 ≤ -3.0 SD were immediately referred because in this sample a large proportion of children have a serious form of CH-T or CCeH, and it was considered that waiting for TSH and TBG results would lead to too much delay in treatment. Because of this approach, a large number of children with TBG deficiency were referred and underwent a diagnostic work-up consisting of TSH and FT4 measurement. For children born at $\leq$36 weeks of gestation with a birth weight $\leq$2500 g, referral is based only on the TSH concentration to avoid unnecessary referral (false-positive results) for a low T4 due to transient hypothyroxinemia of prematurity.

Data retrieval

In the Netherlands, all children with an abnormal neonatal screening result are referred to a pediatric endocrinologist or general pediatrician. TNO Leiden registers all children with an abnormal congenital hypothyroidism screening result and sends out questionnaires to pediatricians to collect data on confirmatory laboratory results and diagnosis. The first questionnaire is sent out shortly after referral; the second questionnaire is sent out when the children are 4 to 5 years of age to reassess the diagnosis. Between 2013 and 2015, a third questionnaire was sent out to pediatricians caring for patients born between 1995 and 2012 with a diagnosis of CCeH based on the two first questionnaires. The third questionnaire requested for further specification of the diagnosis (*e.g.*, whether the child has isolated CCeH or MPHD). MPHD was defined as CeH in combination with at least one more pituitary hormone deficiency.

Using the TNO database, we analyzed screening TSH concentrations and the first diagnostic/pretreatment TSH concentrations of all children diagnosed with permanent CCeH between 1 January 1995 and 13 December 2012. Because children with normal screening results are not referred for diagnostic work-up, data from these children were not available. Results of children with false-positive screening results were available, but this was considered an inappropriate control

group because it may contain children with transient forms of congenital hypothyroidism. Instead, as a control group, we used children who were referred for a low screening T4 but who at diagnostic work-up had TBG deficiency instead of congenital hypothyroidism. Within this group, we used a low cutoff for TBG (TBG concentration <7.5 mg/L serum = <140 nmol/L as measured at diagnostic work-up). All children with a birth weight <2500 g were excluded from the study because TSH concentrations may be slightly higher in premature and low-birth-weight infants.

Screening TSH was measured at five national dedicated screening laboratories with an immunoassay supplied by Perkin Elmer (Waltham, Massachusetts) or Brahms (Waltham, Massachusetts). Assays were standardized using external control samples, allowing use of the same cutoff values. Because children are referred to various hospitals in the Netherlands, pretreatment TSH was measured in various laboratories. Although TSH tests are supplied by different manufacturers, all TSH measurements are performed by immunoassays, with an adult reference range between 0.4 and 4.0 mIU/L in the vast majority of tests.

We hypothesized that in CCeH, TSH values are more often somewhat lower as well as more often somewhat higher (U-shaped distribution) compared with control subjects. Therefore, we categorized screening TSH values as well as diagnostic TSH values. The categorized TSH values of children with CCeH and control subjects were compared. Furthermore, we hypothesized that TSH concentrations in children with CCeH within the framework of MPHD would be higher compared with children with isolated CCeH. Therefore, for the comparison between MPHD and isolated CCeH we did not categorize the TSH values. For categorized data, we used Pearson's χ^2 test or, in case of expected counts <5, we used Fisher's exact test. For continuous data, the Mann-Whitney *U* test was used. A *P* value <0.05 (two-sided) was considered statistically significant.

To test whether FT4 levels may help distinguish CCeH from mild CH-T with comparable TSH levels, we compared FT4 levels of the children with CCeH with FT4 levels of children with CH-T who had TSH levels in the same range as children with CCeH.

The use of the TNO database was in accordance with the Privacy Regulations of the Privacy Committee of the Dutch CH Screening Board.

Results

Between 1995 and 2012, 131 children with CCeH were detected by neonatal screening, 383 children were diagnosed with TBG deficiency, and 1022 children were diagnosed with CH-T. After excluding children with a birth weight <2500 g, the groups consisted of 120 children with CCeH (isolated CCeH, n = 50; MPHD, n = 70) and 350 control subjects with TBG deficiency. The highest measured diagnostic TSH in CCeH was 12.9 mIU/L. Selection of patients with CH-T was made on the basis of TSH comparable to that of the CCeH group. This resulted in the inclusion of 20 cases of CH-T with a first diagnostic TSH <13 mIU/L. In the MPHD group, besides CCeH, the following patterns of pituitary deficiencies were reported: 1 × growth hormone (GH) + adrenocorticotropin (ACTH) + gonadotropin + vasopressin; 2 × GH + ACTH + vasopressin; 21 × GH + ACTH + gonadotropin; 31 × GH + ACTH; 1 × GH + gonadotropin; 1 × ACTH + gonadotropin; 5 × GH; 5 × ACTH; 1 × gonadotropin; two missing data. In 39 cases, information on gonadotropin deficiency was missing or reported as not available. Screening TSH was measured at a median age of 5 days (interquartile range, 2 days), and the first diagnostic TSH and FT4 were measured at a median of 9 days (interquartile range, 5 days). Screening TSH was missing in two cases of CCeH, in one case of MPHD, and in eight control subjects. Diagnostic TSH was missing in one case of MPHD. Diagnostic FT4 was missing in one case of CCeH and in one case of MPHD.

There was a higher percentage of lower (<3 mIU/L serum) as well as higher (≥7 mIU/L serum) screening TSH values in neonates with CCeH compared with the control group. However, these screening TSH concentrations were not statistically significantly different in the CCeH group compared with the control group (Fisher exact test; *P* = 0.055) (Table 1). TSH values at screening were not significantly different in the subgroup of isolated CCeH (median, 3 mIU/L serum) compared with the subgroup with MPHD (median, 3.5 mIU/L serum) (Mann-Whitney *U* test; *P* = 0.568). Diagnostic TSH values were statistically significantly different between the total CCeH group and the control group (Fisher exact test; *P* = 0.037), with somewhat higher percentages of low and (rather) high TSH values in the CCeH group (Table 2; Fig. 1). In a subgroup analysis of MPHD vs isolated CCeH, diagnostic TSH values were higher in the subgroup of MPHD (median, 4.4 mIU/L serum) than in the subgroup of isolated CCeH (median, 3.1 mIU/L serum) (Mann-Whitney *U* test; *P* = 0.004). The distribution of TSH values in MPHD compared with isolated CCeH

Table 1. Screening TSH Concentrations in Children With CCeH, Including Subgroups With Isolated CCeH and CCeH Within the Framework of MPHD and a Control Group Consisting of Children With TBG Deficiency

TSH (mIU/L)	Total CCeH (n = 117)	Isolated CCeH (n = 49)	MPHD (n = 68)	Control Group (n = 342)
<3	35 (29.9%)	17 (34.7%)	18 (26.5%)	97 (28.4%)
3–6.9	77 (65.8%)	29 (59.2%)	48 (70.6%)	227 (66.4%)
7–10.9	1 (0.9%)	1 (2.0%)	0	15 (4.4%)
11–14.9	4 (3.4%)	2 (4.1%)	2 (2.9%)	2 (0.6%)
≥15	0	0	0	1 (0.3%)

Table 2. First Diagnostic TSH Concentrations in Children With CCeH, Including Subgroups With Isolated CCeH and CCeH Within the Framework of MPHD and a Control Group Consisting of Children With TBG Deficiency

TSH (mIU/L)	Total CCeH (n = 119)	Isolated CCeH (n = 50)	MPHD (n = 69)	Control Group (n = 350)
<0.5	3 (2.5%)	2 (4%)	1 (1.4%)	2 (0.6%)
0.5–2.5	33 (27.7%)	19 (38%)	14 (20.3%)	69 (19.7%)
2.6–5	49 (41.2%)	18 (36%)	31 (44.9%)	198 (56.6%)
5.1–7.5	28 (23.5%)	11 (22%)	17 (24.6%)	64 (18.3%)
7.6–10	4 (3.4%)	0	4 (5.8%)	11 (3.1%)
>10	2 (1.7%)	0	2 (2.9%)	6 (1.7%)

was shifted to the right, with the highest TSH values in the MPHD group (Table 2; Fig. 2). Magnetic resonance imaging data were not systematically stored in the database but were available in 36 cases (32 patients with pituitary malformations, three patients with a normal pituitary anatomy, and one patient with uncertain pituitary anatomy). We did have information on the diagnosis of the six children with the highest TSH concentrations (>7.5 mIU/L) at first diagnostic work-up (Table 3). Five of these children had a pituitary malformation, and one had CHARGE syndrome without further data on pituitary anatomy.

In Fig. 3, TSH and FT4 values of the 20 patients with mild CH-T (red) are depicted together with values in isolated CCeH (green) and MPHD (blue). Because distinguishing mild CH-T and CCeH may be difficult and misclassification is possible, we used the available data in the TNO database to review the CH classification. Of the 20 patients with mild CH-T (red circles), the diagnosis CH-T seemed valid in 14 cases based on either additional diagnostic blood tests, imaging (thyroid ultrasound and/or scintigraphy), or available follow-up data at 5 years of age. However, in five cases (solid red dots), we were not sure about the diagnosis. At initial diagnosis and at follow-up around 5 years, these cases were reported to be CH-T by their treating pediatricians. However, in cases 1, 2, and 3 (solid red dots 1, 2, and 3), the available data did not allow for distinction between CCeH and CH-T, and in cases 4 and 5 (solid red dots 5 and 6), CCeH seemed to be more likely. Therefore, among cases 1 through 5, some may be misclassified as CH-T.

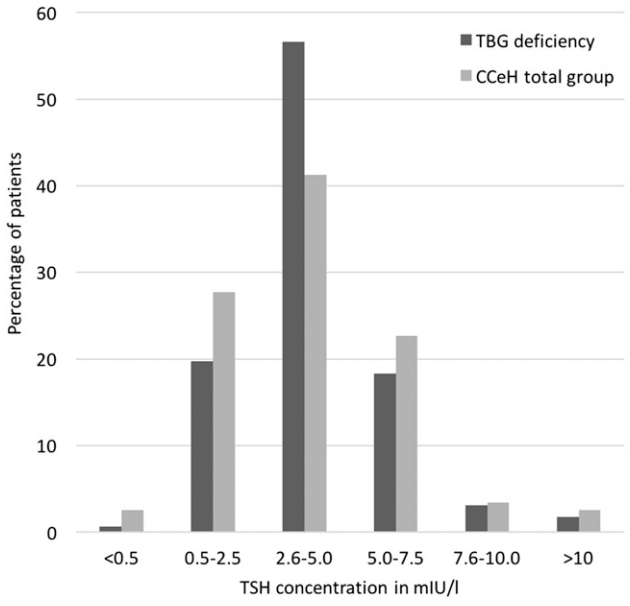

Figure 1. Distribution of the first diagnostic TSH concentrations in the total CCeH group (isolated + MPHD) and TBG deficiency as control group.

FT4 levels were significantly lower in CCeH compared with mild CH-T (Mann-Whitney *U* test; $P < 0.0005$). Within CCeH, FT4 levels were significantly higher in isolated CCeH compared with MPHD (Mann-Whitney *U* test; $P = 0.008$).

Discussion

In this study, we found serum TSH concentrations in neonates with CCeH to be significantly different from TSH concentrations of neonates with TBG deficiency at the time of the first diagnostic laboratory testing after referral for an abnormal neonatal screening result. Compared with neonates with a normally functioning HPT axis, neonates with CCeH had higher percentages of low and high TSH values, whereas the percentage of "normal" values was lower. The same pattern between the groups was found for TSH values based on screening. However, this difference was not statistically significant. Further analyses showed that the higher percentage of low TSH serum values could be mainly attributed to neonates with isolated CCeH, whereas the higher percentage of high values could be mostly attributed to neonates with CCeH within the framework of MPHD. Both findings supported our hypotheses. The highest measured diagnostic TSH in CCeH was 12.9 mIU/L. FT4 levels were significantly lower in CCeH compared with CH-T, with comparable TSH values.

CCeH is characterized by suboptimal thyroid hormone production due to insufficient stimulation by TSH of an otherwise normal thyroid gland (1). The TSH insufficiency may be due to hypothalamic or pituitary pathology leading to a qualitative (*i.e.*, decreased bioactivity) or

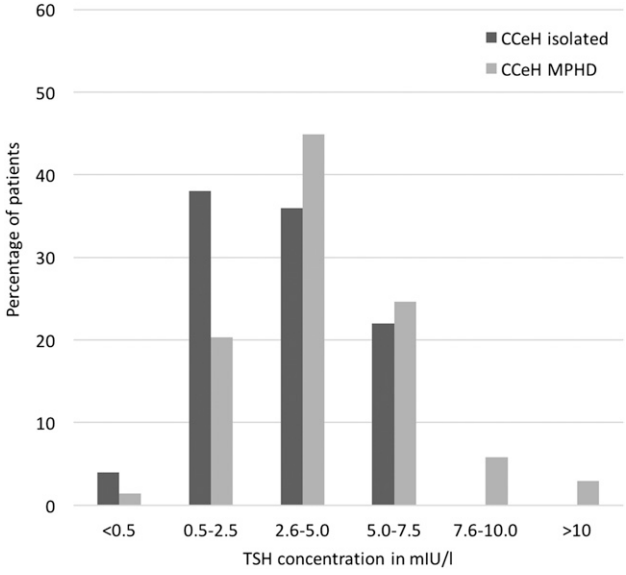

Figure 2. Distribution of the first diagnostic TSH concentrations in the isolated CCeH group and in the CCeH within the framework of the MPHD group.

quantitative defect in TSH production. TSH deficiency can be isolated if the defect is limited to thyrotroph function (isolated CCeH) or may be associated with MPHD (19, 20). Isolated CCeH is less common and may be caused by mutations in the TRH receptor gene (*TRHR*), the thyroid-stimulating hormone β-subunit gene (*TSHB*), and the recently described *IGSF1* and *TBL1X* genes (10–13, 15, 16). In the majority of cases, CCeH occurs with other pituitary hormone deficiencies (9, 19, 20). Various genetic defects in transcription factors involved in pituitary development and differentiation have been described (*POU1F1*, *PROP1*, *HESX1*, *LHX3*, *LHX4*, *OTX2*, *SOX3*, *GLI2*), but in most cases of MPHD the cause is unknown (21, 22). In the majority of these cases, a pituitary malformation is seen on magnetic resonance imaging consisting of an absent or thin pituitary stalk and a hypoplastic anterior and ectopic posterior pituitary lobe, also known as PSIS (9, 18). In these cases, the pituitary stalk malformation results in a lack of hypothalamic TRH stimulation, resulting in impaired TSH glycosylation and secretion. In our study, TSH concentrations were higher in patients with MPHD and CCeH compared with patients with isolated CCeH, suggesting that in the MPHD group the TSH deficiency was more often of hypothalamic origin than in the isolated CCeH group. We did not have information on possible genetic etiologies of the isolated CCeH cases. In general, TSH values in genetic forms of isolated CCeH have been reported to be undetectable to normal. Isolated CCeH due to *TRHR* gene defects have only been described in three cases, and in these cases TSH was low/normal (10, 11). In *TSHB* gene defects, TSH concentrations are usually undetectable but may be low/normal due to bioinactive TSH (12–14). In the newly described genetic forms of X-linked CeH, *IGSF1* and *TBL1X* gene defects, TSH concentrations were reported to be within normal reference ranges (15–17).

Although isolated CCeH is reported to be less frequent than CCeH within MPHD, in our study population the percentage of patients with isolated CCeH was relatively high (50/119 = 42%). Because diagnosing isolated CCeH relies solely on finding too low FT4 concentrations and because proper neonatal reference values for FT4 are missing, we cannot rule out misinterpretation and overrepresentation of these patients. However, it is likely that the high prevalence of isolated CCeH is the result of the unique Dutch neonatal screening program.

The difference in TSH concentrations between MPHD with CCeH and isolated CCeH patients was only clearly detected in the first diagnostic TSH measurements and not in the neonatal screening measurements. A probable explanation for this is that screening TSH is performed on blood samples collected on filter paper from a heel puncture with a larger measurement variation than TSH measurements performed in a venous blood sample. The larger measurement error leads to a lower statistical power to detect differences in screening TSH levels compared with venous TSH levels between the subgroups.

Two children with CCeH within the framework of MPHD had TSH concentrations >10 mIU/L, with the highest measured screening TSH of 14 mIU/L and a pretreatment value of 12.9 mIU/L. In these cases, TSH could have been (or may also be) interpreted as suggestive for mild CH-T. This emphasizes the importance of careful

Table 3. TSH and FT4 Concentrations at First Diagnostic Work-Up in Six Individuals With the Highest TSH Concentrations, Together With Their Diagnosis

Patient No.	TSH (mIU/L)	FT4 (pmol/L)[a]	Diagnosis
1	7.8	10.1	CHARGE syndrome
2	8.3	5.7	PSIS
3	8.8	8.1	PSIS
4	9.0	5.9	Septo-optic-dysplasia
5	10.2	7.8	PSIS
6	12.9	10.1	PSIS

[a]Divide by 12.87 to convert pmol/L to ng/dL.

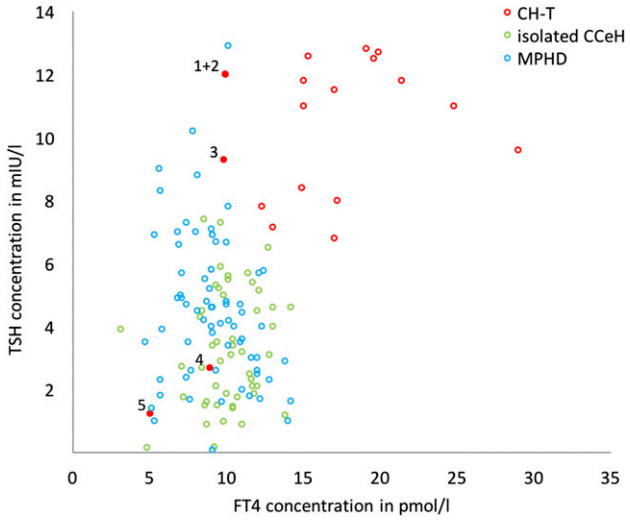

Figure 3. First diagnostic TSH and FT4 concentrations in isolated CCeH (n = 49; green circles), in CCeH within the framework of MPHD (n = 69; blue circles), and in mild CH-T (TSH <13 mIU/L) (n = 20; red circles and red solid dots). Numbered red solid dots are cases possibly misclassified as CH-T.

interpretation of slightly elevated TSH levels. Because proper reference values for FT4 in the neonatal period are not available, diagnosing CCeH remains a challenge, especially in cases of isolated TSH deficiency. FT4 levels in CCeH were on average lower compared with FT4 in CH-T with TSH values in the same range of those with CCeH. If FT4 levels are below the reference range, CCeH must be considered. Additional analysis (*e.g.*, thyroid imaging, pituitary function testing) and careful monitoring of thyroid function tests in the first months of life may provide evidence for a more certain diagnosis.

Our finding of TSH values well above the reference range is in line with reports of TSH values reported in cases of acquired CeH (4–6). In a report on a 2-year Dutch cohort of CCeH (April 1994 to April 1996) in 19 Dutch children with CCeH, first diagnostic TSH concentrations ranged from <0.05 to 10 mIU/L, similar to our results (9). We did not find any reports specifying TSH values in isolated CCeH vs MPHD.

Strengths of our study are that data were extracted from a unique, large national cohort of children with CCeH detected by neonatal screening and that, by sending out a third questionnaire, we verified the diagnosis of permanent CeH at a later age, with the oldest patients being 20 years of age. Furthermore, we were able to include a control group with normal HPT axis because in TBG deficiency only total T4 levels are low and the HPT axis is normal, with normal serum FT4 and TSH concentrations.

A limitation of our study was that the CCeH and CH-T diagnoses were made by a rather large group of pediatricians. Although it seems reasonable to assume that for MPHD diagnoses are reliable, this is less certain for isolated CCeH and mild CH-T, so misinterpretation and misclassification cannot be ruled out. Nonthyroidal illness (accompanied by low TSH and FT4) may also be a differential diagnosis in these cases.

In summary, TSH values in CCeH are more often lower or higher compared with control subjects. TSH concentrations were higher in CCeH within the framework of MPHD compared with isolated CCeH, probably indicating the hypothalamic origin of the hypothyroidism in most patients with MPHD. Patients with CCeH may have TSH concentrations >10 mIU/L. FT4 levels were lower in CCeH compared with CH-T and may be helpful in distinguishing CCeH from mild CH-T.

Acknowledgments

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