Congenital Hypothyroidism With Eutopic Thyroid Gland: Analysis of Clinical and Biochemical Features at Diagnosis and After Re-Evaluation

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Context: In recent years changes in screening strategies for congenital hypothyroidism (CH) led to an increased detection of mild forms of CH, associated with eutopic thyroid gland.

Objectives: We aimed to determine the clinical evolution of CH with eutopic thyroid gland and to find out prognostic factors at diagnosis and follow-up.

Patients and Methods: We retrospectively analyzed a group of 84 children with CH and eutopic thyroid gland treated at our institution. They all underwent clinical re-evaluation after the age of 3, based on thyroid function testing after L-thyroxine therapy withdrawal, thyroid ultrasonography, and ^{123}I scintigraphy with perchlorate discharge test. Genetic analysis was performed in selected cases.

Results: At re-evaluation, 34.5% of patients showed permanent hypothyroidism and needed L-thyroxine reintroduction, 27.4% had persistent hyperthyrotropinemia (TSH 5–10 mU/L), and 38.1% had transient hypothyroidism. Major risk factors for permanent CH were prematurity, first-degree familial history of goiter/nodules, thyroid hypoplasia at diagnosis, and high L-thyroxine requirements at follow-up. Iodine organification defects were found in 29.7% of patients, 30% of whom harbored DUOX2 mutations. TSH receptor gene mutations were found in 8.7% of patients with persistent thyroid dysfunction and negative perchlorate discharge test.

Conclusions: Only one-third of patients with CH and eutopic thyroid gland needed to continue L-thyroxine therapy after re-evaluation. A frequent finding was the persistence of mild hyperthyrotropinemia. The evolution of CH remains difficult to predict, although different clinical features might suggest different outcomes. Mutations in the genes commonly linked to mild forms of CH were documented in a minority of cases. **(*J Clin Endocrinol Metab* 98: 1395–1402, 2013)**

Congenital hypothyroidism (CH) is one of the most relevant endocrine diseases of childhood, potentially leading to permanent mental retardation if not diagnosed and treated in the very early phases of life. In developed countries neonatal screening programs allow a prompt diagnosis and treatment of CH, virtually abolishing severe intellectual disability (1, 2).

In recent years several countries reported a significant increase in CH incidence detected by newborn screening programs, with an almost doubled number of detected

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Abbreviations: b-TSH, basal thyroid-stimulating hormone; CH, congenital hypothyroidism; fT4, free thyroxine; IVF, in vitro fertilization; L-T4, L-thyroxine; PIOD, partial iodide organification defect; Tg, thyroglobulin; TIOD, total iodide organification defect; TPO auto-Ab, antibodies against TPO; TSHR anto-Ab, antibodies against TSH receptor.

cases compared to the usually reported incidence of 1:3000 to 1:4000. In particular, an increased incidence was documented in Italy (1:2200 according to the Italian National Registry of Infants with CH [3]; 1:1446 in Lombardy region [4], 1:1749 in Greece [5], 1:1660 in Massachusetts, USA [6]; 1:1415 in New York, USA [7], and 1:1077 in the United Kingdom [8]). The increasing incidence is mostly explained by changes in screening programs, with the introduction of progressively lower cutoffs to improve sensitivity (9, 10). These strategies led to the detection of a higher number of mild forms of CH, usually associated with a eutopic and normal-shaped thyroid gland (4). However, changes in some demographic factors must be also considered. Indeed, migration trends could have an influence because different ethnicities have different incidence rates of CH, with higher susceptibility in Asian Indian and Hispanic children (11). Another relevant change in modern societies is the increasing number of multiple pregnancies and preterm deliveries, mainly as a result of assisted reproductive technologies, which are considered risk factors for neonatal thyroid dysfunctions (12–14).

In recent years, the increased incidence of mild and potentially transitory forms of thyroid dysfunction started a large debate on the clinical and economical consequences of the new screening strategies adopted by most countries (15–18), in particular, still unsolved issues regarding the increase in the recall of infants with false positive screening tests, and the benefits and costs related to the early detection and treatment of mild forms of thyroid dysfunction. At present, there are no clear data evidence on the long-term clinical outcome of mild hypothyroidism or hyperthyrotropinemia, as far as both intellectual development and metabolism are concerned. Moreover, although novel molecular mechanisms of thyroid function have been reported, the cause of these forms remains largely unknown, and the genotype-phenotype correlations for the most commonly involved genes, DUOX2 and TSH receptor, have been found to be more complex than initially thought, probably due to the existence of genetic and environmental modulators (19, 20).

Despite the presence of CH guidelines (21, 22), the management of mild thyroid dysfunctions still greatly differs between single centers, especially with regard to initial treatment and strategies for clinical re-evaluation to distinguish between transient and permanent forms.

In the present study, we aimed to determine the clinical characteristics, the definitive diagnosis (ie, transient or permanent CH), and the prevalence of the different forms of CH in patients with a normally located thyroid gland. We also analyzed the role played by the changes in neonatal screening programs (ie, the lowering of whole blood TSH threshold to 10 mU/L) on the clinical spectrum of CH in this population.

The final goal is the definition of more appropriate and personalized follow-up protocols for these children, and consequently, better exploitation of clinical and economical resources.

Patients and Methods

All patients of our cohort were born between 1999 and 2005 in Lombardy, a region with 9 642 406 inhabitants at census 2008, characterized by a borderline mild-iodine deficiency, that has its own newborn screening program, centralized in the Laboratory for Neonatal Screening in Milan. The Screening Center adopts a primary TSH method. The basal thyroid-stimulating hormone (b-TSH) threshold level was lowered from 20 mU/L to 12 mU/L in 1999, and then to 10 mU/L in 2002 (4).

In this period a total of 433 patients with positive CH screening were put on L-thyroxine (L-T4) treatment, 289 of them showing a normally located thyroid gland. One hundred sixty-six patients with CH and eutopic thyroid gland were referred to our clinic (Pediatric Unit, San Raffaele Hospital, Milan, Italy). We retrospectively analyzed a group of 84 consecutive patients who underwent clinical re-evaluation after the age of 3, based on thyroid function testing after L-T4 therapy withdrawal, thyroid ultrasound, and radioiodine scanning (^{123}I) with perchlorate discharge test. Patients with Down syndrome or other major syndromes were excluded.

CH diagnosis

Newborns with serum TSH persistently higher than 10 mU/L and normal or lo free thyroxine (fT4) values were diagnosed with CH. L-T4 therapy was introduced at initial doses of 8–14 μg/kg per day, according to the degree of hypothyroidism. Initial dose was lower than 8 μg/kg per day in 8 patients, who started treatment after the second month of life, due to fluctuating TSH values. Serum thyroglobulin (Tg), antibodies against TPO (TPO auto-Ab), and TSH receptor (TSHR anto-Ab) were measured. The presence of a eutopic normal-shaped thyroid gland was ascertained with ultrasound, performed by the same radiologist with experience in pediatric thyroid ultrasonography. The definition of thyroid size was based on the measurement of the anterior-posterior diameter of the gland, as compared to measurements on a healthy population in our clinic. Four patients, who did not perform ultrasound, underwent ^{99}Tc scanning. Clinical data on gestational age, delivery, maternal or neonatal history of iodine exposure, congenital malformations, and familial thyroid disease were collected. All parents underwent thyroid function testing (ie, TSH and fT4), including the measurement of maternal thyroid auto-antibodies.

All patients underwent periodical investigations at our center to ascertain the correct L-T4 dosage and the regular psychophysical development.

Clinical re-evaluation protocol

Between the ages of 3 and 6 years, L-T4 therapy was withdrawn for 4 weeks in all patients, and thyroid function was retested (ie, TSH, fT4, free triiodothyronine, Tg, TPO, and TSHR anto-Ab). Patients with TSH values >15 mU/L immediately re-

sumed L-T4 therapy. Patients showing normal or slightly elevated TSH elevation (ie, 5–15 mU/L) underwent repeated hormonal assessments at regular intervals to determine the TSH trend.

On the basis of definitive TSH values at follow-up, after therapy withdrawal, patients were divided into 3 groups:

- Permanent CH, if serum TSH steadily increased over 10 mU/L, requiring treatment reintroduction.
- Persistent hyperthyrotropinemia, defined as moderate serum TSH elevation (5–10 mU/L) during follow-up; treatment was restarted in selected cases with partial iodide organification defect (PIOD) and/or familial history of goiter/thyroid nodules.
- Transient CH, defined as normal thyroid function with normal serum TSH levels (<5 mU/L) after therapy withdrawal and during the subsequent follow-up of at least 1 year.

Thyroid size was reinvestigated by thyroid ultrasound, performed by the same radiologist. Iodine organification defects were detected by ^{123}I scintigraphy with perchlorate discharge test (23). In particular, a reduction >10% of the ^{123}I uptake levels at 2 hours after oral administration of sodium perchlorate was considered positive for an iodide organification defect. Patients with iodide discharge of 10% to 90% were considered to have a PIOD, whereas patients with iodide discharge greater than 90% were considered to have a total iodide organification defect (TIOD).

The genetic analyses were performed in most patients, according to the perchlorate discharge test results (19). In 20 of 24 patients with PIOD, genetic analysis of DUOX2 and DUOXA2 genes were done. The only patient with TIOD underwent genetic analysis of TPO. In 23 of 32 patients who were negative for iodine organification defects but showed persistent thyroid dysfunction, TSH-receptor gene was analyzed.

Statistical analysis

Comparison between groups was performed using Kruskal-Wallis test for nonparametric values. $P < .05$ was considered statistically significant.

Results

A total of 84 consecutive patients (45 males, 39 females) were analyzed in this study. A history of prematurity (n = 19, 27–36 weeks gestation) was present in 22.6% of cases and 13% were dizygotic twins (n = 11, only in 1 case concordant for thyroid disease). Three children were born from in vitro fertilization (IVF) and 2 of them were twins.

Cesarean delivery occurred in 47.6% of patients, with the prevalence decreasing to 40% if prematurity and twins delivery are excluded.

Associated malformations were present in 14.3% of patients (n = 12), with a predominance of cardiac defects (10 cases, among which 4 were severe and 1 was associated with esophageal atresia); hypospadia was found in 2 cases.

At re-evaluation, 34.5% of patients (n = 29) showed permanent hypothyroidism; 27.4% of patients (n = 23) showed persistent hyperthyrotropinemia, and 38.1% of patients (n = 32) showed transient hypothyroidism.

Correlations between definitive diagnosis and the clinical and instrumental features at diagnosis and re-evaluation were investigated.

Analysis of clinical and biochemical features at diagnosis (Table 1)

Differences in the rate of cesarean section, sex, neonatal screening values, serum TSH, and fT4 at diagnosis were not significant between the 3 groups of patients. Preterm children showed a high rate of permanent CH (52.7%), representing 34.5% of the cohort with permanent CH. All patients born after IVF had permanent CH.

Among laboratory parameters at diagnosis, we found higher levels of Tg in patients with transient hypothyroid-

Table 1. Clinical and Biochemical Features at CH Diagnosis: Comparison Between Patients With Permanent CH, Persistent Hyperthyrotropinemia, and Transient CH

	Permanent CH (n = 29; 34.5%)	Persistent Hyperthyrotropinemia (n = 23; 27.4%)	Transient CH (n = 32; 38.1%)
Sex: F/M	13/16	10/13	17/15
Preterms (<37 gestational weeks)	10 (34.5%)	4 (17.4%)	5 (15.6%)
Caesarean section	15 (51.7%)	10 (43.5%)	15 (46.9%)
Twins	5 (17.2%)	2 (8.7%)	4 (12.5%)
Congenital malformations	4 (13.7%)	4 (17.4%)	4 (12.5%)
TSH screening (mU/L)	15 (5.2–135)	13.3 (10–54)	14.5 (10–85)
Serum TSH (mU/L) (n.v. 0.5–6.3)	22.6 (10–331)	58.6 (11.5–601)	42.5 (10.8–418)
Serum fT4 (ng/dL) (n.v. 1.5–2.4)	1.1 (0.1–1.7)	0.7 (0.1–1.8)	1 (0.2–1.9)
Thyroglobulin (ng/mL) (n.v. 10–250)	107 (1–1469)[a]	423 (18.7–2250)	423.5 (24–2029)[a]
TSHR auto-Ab		0	3 (9.4%)
TPO auto-Ab	0	1	1
First-degree familial thyroid disease/thyroid nodules	10 (34.5%)/4	6 (26.1%)/2	6 (18.7%)/1
Ultrasound scan (no. patients)	29	22	29
• Hyperplasic gland (anteroposterior diameter ≥9 mm)	5 (17.2%)	10 (45.4%)	12 (41.3%)
• Hypoplastic gland (anteroposterior diameter ≤5 mm)	4 (13.7%)	1 (4.5%)	0

Abbreviations: F, female; M, male; n.v., normal values. Laboratory values are expressed as median (range).

[a] Significant difference for serum thyroglobulin between patients with permanent and transient CH (ie, $P < .05$, Kruskal-Wallis test).

ism compared to patients with permanent CH ($P < .05$). The comparison of baseline Tg levels between patients with positive and negative perchlorate test was instead not significant (Tg median values: 517.6 vs 500.1 ng/mL).

The presence of familial thyroid disease in first-degree relatives was more frequent in persistent thyroid dysfunctions (34.5% of patients with permanent CH and 26% of patients with persistent hyperthyrotropinemia), with a high prevalence of thyroid nodules. Only 18.7% of patients with transient CH had first-degree relatives with thyroid disease, mainly represented by autoimmune thyroiditis.

At thyroid ultrasound the finding of a hypoplastic thyroid gland was correlated with permanent hypothyroidism (no patients with hypoplastic gland harbored transient hypothyroidism); this data were also confirmed by the selective analysis of term children, excluding the potential confounding factor of prematurity. The common finding of a hyperplasic thyroid gland was equally present in transient CH and in persistent hyperthyrotropinemia (41.3% and 45.4%, respectively) and was less frequent in patients with permanent CH (17.2%).

Analysis of clinical and biochemical features at re-evaluation (Table 2)

Mean age at treatment withdrawal was 4 years in all groups of patients. There was no evidence that different ages could influence thyroid function at treatment withdrawal.

No difference in development or growth was found among the 3 groups of patients.

We found significant differences in the mean L-T4 dose before withdrawal among the 3 groups ($P < .001$). In particular, all patients with permanent CH had an L-T4 requirement above 2 μg/kg per day.

Four patients with permanent CH had extremely low fT4 (ie, <0.3 ng/dL) after treatment withdrawal (13.8%). In addition, 7 patients showed fT4 values in the lower normal range (0.7–0.9 ng/dL). All patients with persistent hyperthyrotropinemia and transient CH had normal fT4 values.

Thyroid ultrasound scan findings were different according to the different diagnoses, although a hypoplastic gland was a frequent finding in all 3 groups, probably as a consequence of L-T4 replacement therapy. In particular, hyperplasic glands at re-evaluation were associated with permanent CH: this was documented in 3 patients with severe hypothyroidism, characterized by elevated serum TSH (60–200 mU/L) and low fT4 (0.1–0.9 ng/dL) at diagnosis, L-T4 requirements above 3 μg/kg per day during follow-up, and marked TSH elevation at therapy withdrawal (81.6–200 mU/L). One patient harbored a Tg defect; 1 patient had a confirmed TPO gene mutation, whereas the third patient showed an organification defect without known mutations (perchlorate washout of 20%, negative analysis for DUOX and DUOXA mutations). The former 2 patients had a familial history of thyroid nodules.

The perchlorate discharge test was positive for iodide organification defects in 29.7% of patients (n = 25): 1 case of TIOD and 24 cases of PIOD. Among them, 40% of cases showed permanent CH at re-evaluation; 36% had persistent hyperthyrotropinemia, and 24% had transient CH. We found no significant correlations between hormonal data and the presence of iodide organification defect. The only patient with TIOD underwent genetic analysis of the TPO gene, which resulted positive for biallelic mutations. In 20 of 24 patients with PIOD, genetic analysis of DUOX2 and DUOXA2 genes was performed, showing mutations of DUOX2 in 6 cases (30%): 1 patient showed permanent CH; 4 patients developed persistent hyperthyrotropinemia, whereas 1 patient showed complete normalization of thyroid function.

Table 2. Clinical and Biochemical Features at CH Re-evaluation: Comparison Between Patients With Permanent CH, Persistent Hyperthyrotropinemia, and Transient CH

	Permanent CH (n = 29; 34.5%)	Persistent Hyperthyrotropinemia (n = 23; 27.4%)	Transient CH (n = 32; 38.1%)
L-Thyroxine dose (μg/kg/d)	2.15 (1–3.7)[a]	1.64 (0.86–2.62)[a]	1.35 (0.4–2.55)
Serum TSH (mU/L) (n.v. 0.25–5)	10.83 (4.4–126.5)	6.95 (3.67–10.4)	3.7 (1.47–7.86)
Serum fT4 (ng/dL) (n.v. 0.7–1.7)	1.09 (0.2–1.38)	1.20 (0.95–1.61)	1.33 (0.93–1.83)
Ultrasound scan			
• Hyperplasic gland (anteroposterior diameter ≥12 mm)	3 (10.3%)	0	0
• Hypoplastic gland (anteroposterior diameter ≤7 mm)	10 (34.4%)	9 (39.1%)	13 (40.6%)
Iodine organification defect	10 (34.4%)	10 (35.7%)	5 (18.5%)

Abbreviation: n.v., normal values. L-Thyroxine dose is expressed as median (range).

[a] Significant difference for L-thyroxine between patients with permanent and transient CH (ie, $P < .001$, Kruskal-Wallis test) and between patients with permanent CH and persistent hyperthyrotropinemia (ie, $P < .05$).

Table 3. Comparison of Patients With Screening TSH <20 mU/L and Screening TSH ≥20 mU/L: Biochemical Features at CH Diagnosis and Definitive Diagnosis at Re-evaluation

	Screening TSH (<20 mU/L; n = 64; 76.2%)	Screening TSH (≥20 mU/L; n = 20; 23.8%)
Preterms (<37 gestational weeks)	14 (21.8%)	5 (25%)
Serum TSH, mU/L (n.v. 0.5–6.3)	29.8 (10–418)	78.7 (12–601)
Serum fT4 (ng/dL) (n.v. 1.5–2.4)	1.0 (0.2–1.9)	0.7 (0.1–1.7)
Thyroglobulin (ng/mL) (n.v. 10–250)	354 (7.2–2250)	122 (1–1380)
Definitive diagnosis		
• Permanent CH	22 (34.3%)	7 (35%)
• Persistent hyperthyrotropinemia	18 (28%)	5 (25%)
• Transient CH	24 (37.5%)	8 (40%)

Abbreviation: n.v., normal values. Laboratory values are expressed as median (range).

In 23 of 32 patients with negative perchlorate discharge test and persistent thyroid dysfunction, the TSH receptor gene was analyzed. Mutations were found in 2 patients (8.7%), both with hypoplastic thyroid gland at diagnosis and permanent CH.

Comparison between patients with screening TSH <20 mU/L and screening TSH >20 mU/L (Table 3)

b-TSH value at neonatal screening was <20 mU/L in 76.2% of patients (n = 64). Therefore, all these cases would have been missed before 1999, when the screening cutoff in the Lombardy region was lower. Interestingly, serum confirmatory values of these patients often showed severe forms of CH: fT4 was low for age (<1.5 ng/dL; normal values, 1.5–2.4 ng/dL) in 79.7% of cases; serum TSH increased to levels >50 mU/L in 37.5%, and >100 mU/L in 21.8% of cases. The definitive diagnosis at re-evaluation in patients with lower screening TSH values (<20 mU/L) show the same distribution as patients with screening TSH >20 mU/L.

Discussion

Congenital hypothyroidism has evolved into a condition whose manifestations range from severe to mild (16). International guidelines recommend to withdraw L-T4 therapy after the age of 3 years in case of CH with eutopic thyroid gland, to evaluate the persistence of the condition (22). Currently, however, no specific indications exist on how to perform diagnostic re-evaluation and the follow-up of these patients, mainly due to the lack of large-scale, long-term studies on milder forms of CH.

In our study we analyzed the clinical and biochemical features and the outcome of 84 patients with CH and eutopic thyroid gland, followed at a single center, who systematically underwent re-evaluation after the age of 3 years. Remarkably, more than one-third of patients (38.1%) showed a complete normalization of thyroid function after treatment withdrawal, confirmed after at least 1 year of follow-up. This prevalence of transient hypothyroidism is similar to that reported by Gaudino et al (24) and highlights the importance of diagnostic re-evaluation to avoid unnecessary treatment later in life. In the remaining two-thirds of patients, however, a persistence of thyroid dysfunction was demonstrated: 34.5% of cases showed permanent CH, requiring L-T4 therapy reintroduction, and 27.4% of cases showed persistent hyperthyrotropinemia.

In our clinical experience it is crucial to monitor TSH values closely in the first months after treatment withdrawal, which occurred at a mean age of 4 years in the present cohort. Indeed, slightly elevated TSH values found immediately after withdrawal can both normalize and increase over time, as well as normal TSH values can increase promptly or at several months from treatment withdrawal. We cannot exclude that a second attempt to withdraw treatment at the end of pubertal development could lead to new information on the progression of thyroid dysfunction.

It is interesting to observe that patients with screening b-TSH values <20 mU/L showed the same distribution of clinical outcome after therapy withdrawal as patients with higher screening values. The former cases represent more than two-thirds of our cohort (76.2% of patients) but they could be considered a "newly detected population," as they would have been missed in our region before 1999. At CH diagnosis, 79.7% of the patients with b-TSH <20 mU/L showed low levels of fT4 for age. At re-evaluation 34.3% of those children showed permanent CH, requiring L-T4 treatment, and 28% showed persistent hyperthyrotropinemia, needing periodical thyroid function retesting. Thus, present data support the new screening strategies that introduced lower screening TSH thresholds, leading to an early detection of a large number of patients with persistent thyroid dysfunction. Nevertheless, further long-term studies are needed to establish the benefits of treatment of milder cases of CH also considering that no data

are available about the intellectual outcome of these patients (18).

The overview of our population discloses a remarkable number of preterm children (22.6%) compared to the general Italian population (6.75% in 2009 [25]), with a high prevalence of permanent CH in this category of patients (52.7%). Certainly, as reported in literature, thyroid dysfunctions in preterm newborns often rapidly improve spontaneously, without need for hormonal treatment (26). Nonetheless, our work suggests that preterm newborns presenting TSH values >10 mU/L, confirmed over time, develop permanent CH more frequently as compared to term newborns. This is in accordance with data reported by Mengreli et al showing permanent thyroid dysfunction in 83.3% of preterm newborns initially treated for CH (5). The prevalence of twins in our series (13%) was considerably higher than in the normal population (1.5% in Italy [25]). This is concordant with the 3-fold risk of CH in twins described in literature (12). All patients born after IVF, in accordance with data reported by Sakka et al, showed permanent CH (14).

Familial history for thyroid diseases was found to be frequent in all 3 categories of patients: a deeper analysis showed that maternal autoimmune disease, mainly with antibodies against TSH receptor, was related to transient hypothyroidism, whereas familial cases of goiter and nodular disease were more frequently recorded in children with permanent CH.

In addition, we looked for clinical or biochemical indexes possibly predicting the persistence of hypothyroidism by analyzing the features at diagnosis (Table 4). We did not find significant differences in hormonal values between the 3 groups, with the exception of higher Tg levels in patients with transient CH and persistent hyperthyrotropinemia, compared to permanent CH. This could be explained by the fact that Tg usually rises in response to transient interferences in thyroid hormone synthesis or in the case of iodine organification defects. As recently reported by different authors, DUOX2 mutations typically cause transient CH or develop into mild hyperthyrotropinemia after the first years of life (27–29).

The ultrasonographic finding of a hypoplastic thyroid gland at diagnosis was related to permanent CH. Thyroid ultrasound at diagnosis is therefore an important diagnostic tool that can also hint to the patient's prognosis. In contrast, the finding of a hypoplastic gland at re-evaluation is frequent and without clinical relevance. Thyroid volume is probably influenced by replacement therapy, most patients showing a normal or hypoplastic gland soon after L-T4 withdrawal. On the other hand, the finding of a hyperplasic gland at re-evaluation was invariably associated with severe permanent hypothyroidism due to iodide organification defects. In this subgroup of patients, the administration of higher L-T4 dosage should be considered, to keep TSH levels below 2 mU/L, preventing or at least limiting, thyroid enlargement.

Table 4. Risk Factors for Permanent CH

- Prematurity
- First-degree relatives with history of goiter/nodules
- Thyroid hypoplasia at diagnosis (anteroposterior diameter ≤5 mm)
- High L-thyroxine requirements at re-evaluation (>2 μg/kg/d)
- Thyroid hyperplasia at re-evaluation

Another feature associated with the permanence of CH was found to be the L-T4 dose at the time of withdrawal, as already reported by other authors (30). In our study, all patients with permanent hypothyroidism had L-T4 requirements higher than 2 μg/kg per day.

An important issue raised by our analysis regards the utility of perchlorate discharge test at re-evaluation. In our population, it helped to detect an organification defect in approximately 30% of patients, most them (80%) showing persistent thyroid dysfunctions. Because those cases showed a wide clinical variability, probably due to the different genetic background, we believe that it would be difficult to select a candidate category for the test. Thus, our indication would be to perform the perchlorate discharge test only in specialized centers with the main aim to target the genetic analyses.

Genetic analysis for DUOX and DUOXA, performed in 20 patients with PIOD, showed a mutation of DUOX in 30% of cases. Further studies investigating the correlation between genotype and phenotype are needed to select patients better for DUOX2 analysis. Moreover, the absence of DUOX2 mutations in a significant number of our patients with PIOD suggests that other still unknown molecular mechanisms are responsible for most of these cases.

Another critical point is related to the follow-up of patients with an iodide organification defect and a normal thyroid function (20% of our series): should these children be monitored for the potential risk of goiter in adult age or should they be considered as transient hypothyroid? Also, in this case, a long-term follow-up could clarify the risk and help optimize health resources.

Patients with a persistent thyroid dysfunction and without iodide organification defects were submitted to TSHR gene analysis. We found a mutation in 2 patients (8.7%), both with hypoplastic thyroid gland at diagnosis and permanent CH at re-evaluation. The prevalence of TSHR mutation in our population is similar to that recently reported by Narumi et al (31) and Calebiro et al (32). Interestingly, in most cases of permanent CH, we could not identify the cause of thyroid dysfunction. In fact, 65.6% of patients with negative perchlorate test were negative for a TSHR

gene mutation, indicating that further studies are needed to elucidate mechanisms of thyroid dysfunction in this large cohort of patients.

In conclusion, CH with eutopic thyroid gland is an extremely complex condition, still lacking consensus regarding its diagnosis and treatment. Our study investigated the phenotype of a large cohort of patients, highlighting different aspects that are still a matter of debate. The frequent persistence of thyroid dysfunctions at re-evaluation also in patients with initial low screening TSH values underlines the importance of redefining screening strategies, with special attention toward high-risk categories like preterm newborns. Similarly, a diagnostic re-evaluation after 2 to 3 years of age is needed to identify transient forms of CH. We showed that the examination of thyroid volume at diagnosis and at re-evaluation, as well as the L-T4 requirements at follow-up and the familial history for thyroid disease, can represent useful prognostic data. Genetic defects are certainly responsible for a high percentage of patients with a persistent thyroid dysfunction, even though our knowledge is still limited in this field. Indeed, mutations in the currently known genes linked to mild forms of CH (DUOX and TSHR) explain only a minority of cases. It is likely that CH with eutopic thyroid gland could not be a monogenic disease, and that epigenetic mechanisms and environmental factors could be involved in disease expression. For example, the mild iodine deficiency documented in the Lombardy region may contribute to the elevated incidence of CH with in situ thyroid gland (1:1446) detected in this region. It could be speculated that a more efficient iodine prophylaxis, especially during pregnancy, would reduce the number of patients with transient CH and persistent mild thyroid dysfunctions. Further studies on long-term follow-up and genotype-phenotype correlations are needed to improve our understanding of congenital thyroid dysfunctions and to clarify the best clinical management for these patients.

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