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Hyperplastic Pituitary Gland, High Serum Glycoprotein Hormone α-Subunit, and Variable Circulating Thyrotropin (TSH) Levels as Hallmark of Central Hypothyroidism due to Mutations of the TSHβ Gene*

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ABSTRACT

Inheritable isolated central hypothyroidism (ICH) due to mutations of TSHβ gene has been reported in few patients. For this reason the diagnostic criteria are vague. The disorder is usually characterized by undetectable TSH levels, although low/normal serum TSH, depending on TSH measurement methods, has been documented in some patients. Here we report an Egyptian girl with ICH due to a novel nonsense mutation of the TSHβ gene (Q49X). She was referred at 75 days of age for severe clinical signs of hypothyroidism, whose central origin was documented by normal serum TSH, low free T_4 and free T_3 levels, impaired TSH response to TRH, absence of ^{99}Tc thyroidal uptake, and antithyroid autoantibodies. Ultrasound revealed a hypoplastic thyroid, whereas magnetic resonance imaging showed a hyperplastic pituitary. All other pituitary hormones, including PRL, were normally secreted. A diagnosis of idiopathic ICH was made, and substitutive L-T_4 treatment was started at 81 days of age. At the age of 7 yr the patient had normal thyroid hormone levels, but was severely mentally retarded. Interestingly, the sella computed tomography scan had completely normalized. At 8 yr of age the patient was reinvestigated after 6-week L-T_4 withdrawal. TSH values were highly variable depending on the measurement method used, whereas extremely high levels of circulating free glycoprotein α-subunit were recorded. Despite the fact that mutant TSHβ lacks 60% of the C-terminal amino acid sequence, it forms with the α-subunit a heterodimer with preserved immunoreactivity in some TSH measurement methods, but the mutant heterodimer is completely devoid of bioactivity. In conclusion, high circulating free glycoprotein α-subunit levels, variable TSH levels, and, possibly, hyperplastic pituitary gland are the hallmark of ICH due to mutations of the TSHβ gene. (*J Clin Endocrinol Metab* **86:** 1600–1604, 2001)

INHERITABLE HYPOTHYROIDISM of central origin is the consequence of heterogeneous genetic alterations, all leading to impaired thyroid stimulation. Mutations of genes encoding several transcription factors, such as Pit-1, Prop-1, Hesx1, and Lhx3 (1–5), as well as leptin receptor (6) are possible causes of central hypothyroidism combined with deficiencies of other pituitary hormones. Conversely, congenital isolated central hypothyroidism (ICH) may be the consequence of mutations of either TSHβ (7–14) or TRH receptor (15) genes.

Since the cloning of the TSHβ gene (16, 17), three different TSHβ mutants have been described in several familial settings of congenital ICH. All of the affected patients had homozygous mutations in exon 2 or 3 of the TSHβ gene. Two mutations located in the N-terminal part of the subunit (Q12X and G29R) prevented the assembly of the heterodimer, and the affected patients were biochemically characterized by undetectable circulating TSH concentrations (7–10). On the contrary, heterodimerization was not completely prevented by a particular frameshift mutation (C105Δ) in the TSHβ carboxyl-terminal with a premature stop codon in the "seat-belt region" of TSH (C105Δ,114X), and the affected patients had variable serum TSH concentrations depending on the TSH measurement method employed (11–14). In fact, in noncompetitive assays the ability of anti-TSH monoclonal antibodies to bind epitopes still present on the mutant heterodimer leads to the formation of the "sandwich" and, therefore, to the measurement of certain amounts of TSH. Recently, another form of inherited central hypothyroidism has been recognized as being due to an inactivating mutation of the TRH receptor gene, a situation accompanied by a mild hypothyroid state and normal basal TSH and PRL levels that are unresponsive to TRH stimulation (15).

Recognition of the genetic defect underlying ICH is often difficult due to the low number of cases reported to date and the consequent lack of clear-cut clinical and biochemical diagnostic criteria. ICH with undetectable or low TSH levels is, in general, considered suggestive of TSHβ mutations, in particular when high levels of glycoprotein hormone α-subunit (αGSU) are concomitantly recorded (11, 18). Conversely, resistance to TRH due to TRH receptor mutations is suspected

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on the basis of blunted responses of both TSH and PRL to the TRH test (15).

In all of the above patients, the hypothyroid state is not detected at neonatal screening for congenital hypothyroidism, as the only test used in most centers is TSH evaluation on a dry blood spot (19). The diagnosis is, therefore, delayed, and the hypothyroid state is often severe (20). The delayed start of L-T_4 replacement therapy may result in slight to severe mental and growth retardation (7–15, 18, 20).

Here, we report a girl with congenital ICH carrying a novel homozygous mutation in the TSH β-subunit gene. The data collected along with those previously reported allow delineating the diagnostic criteria of such a rare pituitary-thyroid disorder.

Subjects and Methods

Case report

Early studies. The propositus is an Egyptian girl, normally delivered at term after an uneventful gestation. Her birth weight and length were 2250 g and 46 cm (10th percentile), respectively. She is the first of four sisters. The parents are consanguineous (first cousins). She was referred for feeding problems, jaundice, growth (length, 51 cm), and neuromotor delay at 75 days after delivery. Physical examination showed umbilical hernia, severe hypotonia, large posterior fontanel, hoarse cry, dry skin, and epicanthus. Routine biochemical tests were normal. Thyroid function tests showed severe hypothyroidism in the presence of normal serum TSH (Table 1) and absence of antithyroid autoantibodies, including those against the TSH receptor. TRH administration was followed by an impaired TSH response (basal, 3.4 mU/L; peak, 4.8 mU/L). Other pituitary functions, including PRL, GH, and adrenal axes, were normal.

Interestingly, sella magnetic resonance imaging revealed a hyperplastic pituitary gland (diameters of the adenohypophysis sagittal projection: height, 6 mm; length, 8 mm; coronal projection: width, 11 mm), an unexpected finding in patients with ICH. Moreover, no ^{99}Tc thyroidal uptake was recorded, although ultrasound revealed a hypoplastic thyroid gland. Acoustic evoked potentials showed a modest impairment of hearing function, whereas a roentgenogram revealed the absence of the nucleus of femur proximal epiphyses. The diagnosis of ICH of possible genetic origin was missed, the disease was labeled as idiopathic, and L-T_4 substitutive therapy was started at 81 days of age at a dose of 8.4 μg/kg BW·day.

Follow-up studies. The patient was reevaluated at 21 months of age during L-T_4 therapy (Table 1). She was 78 cm in height (third percentile). The compliance with therapy was judged to be good on the basis of normal circulating levels of free T_4 (FT_4) and FT_3. A sella computed tomography scan showed a completely normalized pituitary size. The GH response to arginine (basal, 2.0 μg/L; peak, 10.0 μg/L) and basal PRL levels (294 mU/L; normal, 100–500) were normal. In that period, the patient's family moved back to Egypt, and subsequent follow-up became difficult. The patient was seen again at the age of 7 yr while receiving L-T_4 treatment. Bone age was delayed (~1 yr), and her growth rate was at the third percentile. An improvement of the neuromotor delay was noted, and hearing function was normalized, but her neurological conditions, including language and visual-spatial abilities, were impaired. Her intelligence quotient (score, 65) was low. Although FT_4 and FT_3 levels were within the normal range, the L-T_4 dose was not adequate for patient age and body weight (2.3 *vs.* an adequate regimen of 3–4 μg/kg BW·day) (21). The inadequacy of substitutive therapy was subsequently confirmed by the finding of α-GSU levels much higher than those found in controls matched for similar circulating levels of pituitary glycoprotein hormones.

One year later, at the age of 8 yr, the patient was reinvestigated at our institutes both before and after L-T_4 withdrawal. Serum TSH was measured by means of several immunometric assays. In addition, free α-GSU and basal and TRH-stimulated PRL levels were tested (Table 1 and Fig. 1). Blood samples for genetic and biochemical studies were obtained from all family members after informed consent of the parents was given.

Immunoassays

Immunoreactive TSH was measured using two second generation methods [enzyme-linked immunosorbent assay (Roche, Mannheim, Germany): sensitivity, 0.05 mU/L; Delfia (Wallac, Inc. Turku, Finland): sensitivity, 0.03 mU/L] and three third generation methods [enzymatic chemiluminescent immunoassay (Roche): sensitivity, 0.007 mU/L; Myria (Bouty, Sesto S. Giovanni, Italy): sensitivity, 0.002 mU/L; AutoDelfia Ultra (Wallac, Inc.): sensitivity, 0.005 mU/L]. The TSH International Reference Preparation 80/558 was the reference preparation for all of these assays. All methods, except the Delfia Ultra kit, are two-site immunoassays based on the sandwich technique, in which two monoclonal antibodies are directed against two separate antigenic determinants on the TSH heterodimer. One monoclonal antibody is directed against a specific α/β conformational antigenic site, and the other is directed against a specific epitope on TSHβ. The Delfia Ultra Kit is based on the use of three monoclonal antibodies conferring a very high specificity to the TSH assay; the capture antibody is directed against an α/β conformational epitope of the TSH heterodimer, whereas the other two monoclonal antibodies are directed against two different antigenic sites of TSHβ subunit (three-site immunoassay). The cross-reactivity of free α-GSU or TSHβ is absent in all of the above TSH immunoassays. Possible

TABLE 1. Hormone parameters, including serum TSH immunoreactivity, in the propositus at diagnosis and along the follow-up

Parameter	75 days (before L-T_4)	21 months (on L-T_4)[a]	7 yr (on L-T_4)[a]	8 yr (on L-T_4)[a]	8 yr (6 weeks after L-T_4 withdrawal)	Normal values	TSH assays
							Two-site immunoassays
TSH (mU/L)	3.74					0.3–5.0	2nd generation ELISA, ES600, Roche
			1.18	2.33	3.46	0.3–5.0	2nd generation IFMA, Delfia, Wallac
		1.16	0.3	1.15	2.55	0.27–4.5	3rd generation ECLIA, Elecsys, Roche
					<0.002	0.25–4.0	3rd generation IRMA, Myria, Bouty
							Three-site immunoassay
			0.01	0.02	0.02	0.24–4.0	3rd generation IFMA, DelfiaUltra, Wallac
α-GSU (μg/L)			4.9	9.4	22.5	0.1–1.0	
FT_4 (pmol/L)	2.6	12.9	14.1	8.2	2.1	9.0–20.0	
FT_3 (pmol/L)	1.1	6.4	4.6	4.0	1.5	4.0–8.0	
Thyroglobulin (μg/L)			1.1		1.2	3–30	
LH (U/L)					0.1	<0.15	
FSH (U/L)					0.3	0.1–1.0	

IRMA, Immunoradiometric assay.

[a] L-T_4 dose: 4.5 μg/kg BW·day at 21 months, 2.3 μg/kg BW·day at 7 yr, 2.0 μg/kg BW·day at 8 yr.

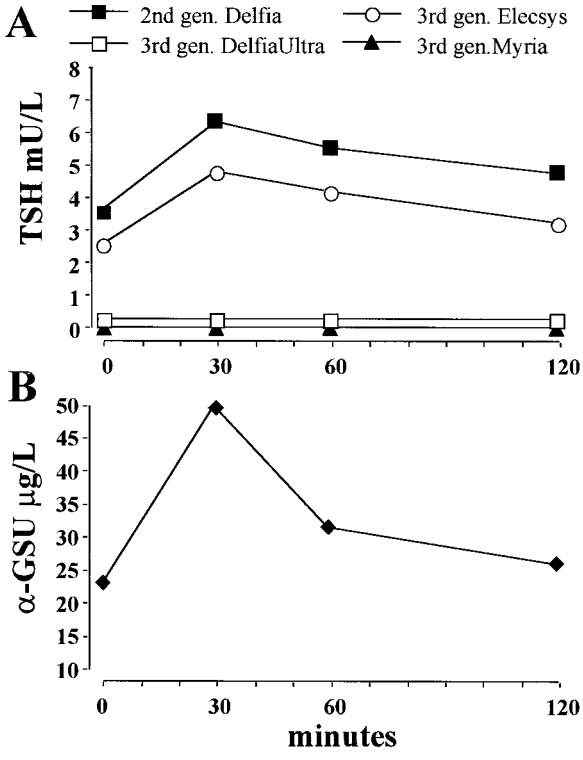

FIG. 1. Results of TRH test (7 μg/kg BW, iv) performed at the age of 8 yr, 6 week after L-T_4 treatment withdrawal. A, Impaired TSH response to TRH measured by means of four different immunometric assays (see *Materials and Methods* and Table 1). B, Exaggerated α-GSU response to TRH, indicating a profound alteration of TSH subunit assembly within pituitary thyrotropes.

interference (leading to an overestimation of serum TSH values) due to the presence in the serum of heterophilic antimouse autoantibodies is prevented by the use of chimeric (mouse-human) antibodies (in the enzyme-linked immunosorbent assay and the enzymatic chemiluminescent immunoassay) and/or the addition of mouse serum to the assay buffer. To exclude methodological interference due to anti-TSH antibodies or other circulating factors cross-reacting with the assay monoclonal antibodies, serum dilution (1:2.5, 1:5, and 1:10) and recovery (8, 16, and 32 mU/L TSH IRP 80/558 were added to the serum samples) tests were carried out by using both second and third generation Delfia assays.

Serum FT_3 and FT_4 levels were measured by direct back-titration methods, using Delfia technology (Wallac, Inc.). Serum levels of α-GSU were evaluated by a sensitive and specific two-site immunoradiometric assay (Biocode, Sclessin, Belgium). Other hormones were evaluated by means of sensitive and specific commercial kits.

DNA sequencing

Genomic DNA was extracted from peripheral whole blood using NUCLEON BACC2 (Amersham Pharmacia Biotech Italia, Cologno Monzese, Italy). The coding region of the TSHβ gene was amplified using primers and PCR conditions previously described (12). PCR was performed in 100 μL reaction solution containing 400 ng genomic DNA, 1.5 mmol/L $MgCl_2$, 40 pmol of each primer, and 0.5 U DNA *Taq* polymerase (Promega Corp., Madison, WI). The purified PCR products were directly sequenced using the Big Dye Terminator kit and the ABI 310 automated sequencer (PE Applied Biosystems, Fosters City, CA).

Results

Hormonal studies

Data collected at the age of 8 yr both before and after L-T_4 substitutive therapy withdrawal showed normal circulating TSH concentrations in most of the noncompetitive immunoassays (Table 1). TSH immunoreactivity was undetectable in two immunoassays, including that showing the highest specificity (Delfia Ultra). In both second and third generation Delfia assays, dilution curves of samples collected after L-T_4 withdrawal were parallel to the standard curve, and a recovery of 100 ± 2% was observed at the TSH recovery test. Together, these findings show the lack of methodological interference in the measurement of TSH concentrations.

After withdrawal of L-T_4 for 6 weeks, the TRH test showed an impaired TSH response in two immunoassays, whereas serum TSH immunoreactivity was always undetectable in the remaining two (Fig. 1). Serum FT_4 (basal, 2.1 pmol/L; at 120 min, 2.5 pmol/L) and FT_3 (basal, 1.5 pmol/L; at 120 min, 1.1 pmol/L) did not increase after TRH administration. Serum thyroglobulin levels were low both before and after L-T_4 therapy withdrawal. Serum α-GSU levels were extremely elevated and showed an exaggerated response to TRH after withdrawal of L-T_4 treatment (Table 1 and Fig. 1). Moreover, the high levels of α-GSU recorded during L-T_4 substitutive therapy indicated that the patient was undertreated, as also documented by the relatively low FT_4 concentrations. Serum PRL was at the upper limit of the normal range in basal conditions (508 mU/L; normal, 100–500) and increased normally in response to TRH injection (PRL peak, 1552 mU/L).

The parents and three sisters of the proband had normal thyroid function tests and α-GSU levels (Fig. 2).

Molecular studies

Direct sequencing of the TSHβ gene showed a homozygous C to T transition in exon 3, at position +205 of the coding sequence (17) (Fig. 2). The mutation leads to a premature stop at codon 69, resulting in the synthesis of the mature mutated protein Q49X (after excision of the signal peptide of 20 amino acids). The mutant Q49X lacks 60% of the C-terminal tail of the mature protein. The parents and 2 sisters were heterozygous carriers of the same substitution (Fig. 2).

Discussion

Congenital ICH due to mutation of the TSHβ gene should be suspected whenever a hyperplastic pituitary gland along with variable TSH circulating levels, impaired TSH response to TRH, and high levels of α-GSU are detected. Normal secretion of the other pituitary hormones, including PRL, rules out other forms of congenital hypothyroidism, such as combined pituitary hormone deficiency (1–6) and possible mutation of the TRH receptor gene (15).

TSHβ mutations located in the amino-terminal part prevent subunit assembly (Table 2). The peptide is truncated after 11 amino acids in the case of the nonsense substitution (Q12X) reported by Dacou-Voutetakis (9), whereas the substitution (G29R) found in the Japanese cases (7, 8, 10) alters the domain called the CAGYC region, which is a highly conserved sequence among the β-subunits of glycoprotein hormones (7, 22, 23). Microinjections of this mutant TSHβ messenger ribonucleic acid in *Xenopus laevis* oocytes lead to the formation of conformationally altered peptides that could not associate with the α-subunit, indicating that the

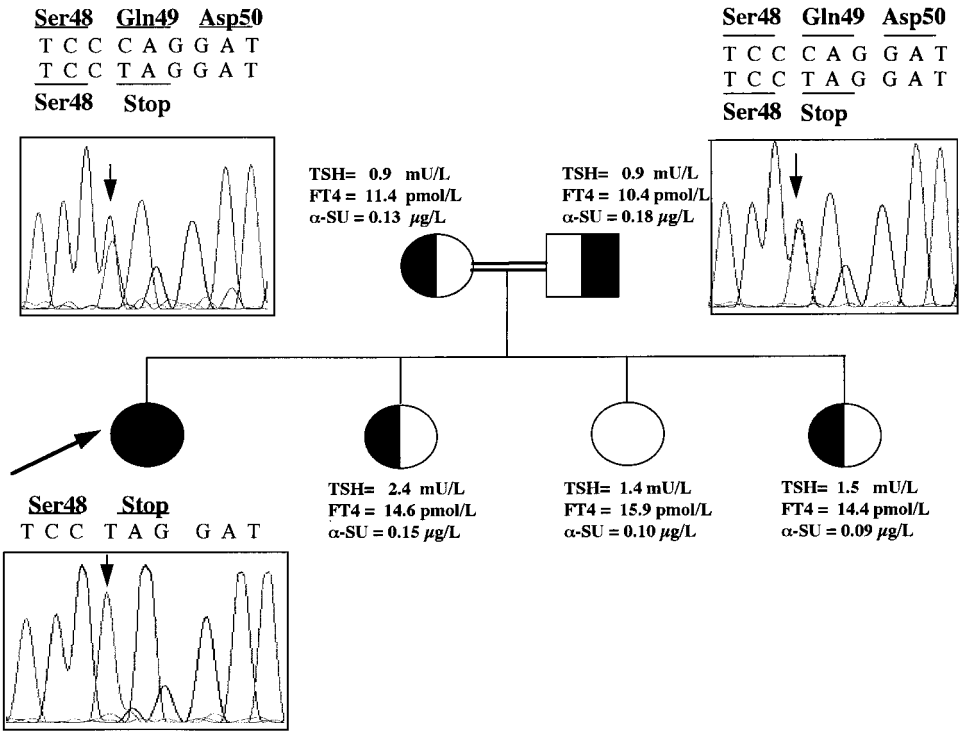

FIG. 2. Schematic pedigree of the family and results of sequence analysis. The C to T transition is located at codon 69 of the TSHβ-coding sequence, leading to the creation of a premature stop at position 49 (Q49X) in the amino acid sequence of the mature protein, *i.e.* after cleavage of the first 20 residues constituting the signal peptide. Note the homozygosity in the patient and the heterozygosity in her unaffected parents and two sisters. Thyroid function test results are within the normal range in all of the relatives.

TABLE 2. List of the mutations of the TSHβ gene causing isolated central hypothyroidism reported to date in the literature

Mutation of TSHβ gene (ref. no.)	Consequences of mutation	Circulating TSH (mU/L)	Circulating α-GSU (μg/L)[a]
G29R (7, 8, 10, 18)	Prevent dimer formation (CAGYC region)	ND	8.0–17.5
Q12X (9)	Truncated TSH β-subunit	ND	
C105Δ, 114X (11–14)	Change of amino acid sequence in the seat belt region	ND–0.8[b] 7.9–14.8[c]	1.2–6.3
Q49X (present case)	Truncated TSH β-subunit	ND–3.7[b]	22.5

Functional consequences of the mutant protein as well as TSH and free α-subunit concentrations are shown. ND, Not detectable.
[a] Measured only in Refs. 11 and 18.
[b] Noncompetitive immunometric assay.
[c] Competitive RIA.

integrity of the CAGYC region is required for correct subunit folding and regular dimer formation (7, 22, 23). As expected, the above mutations are accompanied by undetectable circulating TSH levels. On the contrary, mutations in the carboxyl-terminal part of TSHβ, such as C105Δ,114X (11–14) and Q49X described here, do not prevent formation of the heterodimer (Table 2). Hence, immunoreactive TSH may be detected using both competitive and noncompetitive immunometric assays. In fact, if the mutant TSH possesses the epitopes recognized by the assay monoclonal antibodies, a sandwich may be formed, and a certain amount of TSH may be measured. Indeed, the highest levels of TSH were measured in competitive RIAs using polyclonal antibodies recognizing not only the heterodimer but also the circulating free TSHβ mutant (14). Interestingly, the mutant (Q49X) heterodimer was not recognized by highly sensitive and specific TSH methods, such as the Delfia Ultra Kit, based on the use of three monoclonal antibodies directed against three different epitopes on the TSH heterodimer. Of course, possible methodological interference by circulating anti-TSH antibodies or unknown factors mimicking TSH molecules should be ruled out by appropriate tests, such as dilution and recovery tests.

Whatever the immunoreactivity of circulating TSH, the profound hypothyroid state of the affected patients indicates that the mutant heterodimer is totally unable to stimulate TSH receptor, thus confirming that the C-terminal domain of TSH β-subunit is required for conferring biological activity to TSH. This view was confirmed by the low thyroglobulin levels on and off L-T_4 treatment as well as the absent responses of free thyroid hormones to endogenous TRH-stimulated TSH. On the basis of the crystallographic structure of CG (24), a disulfide bond between C_{19} and C_{105} in the TSH β-subunit is predicted to form the "buckle" of the "seat belt" that surrounds the common α-subunit and maintains the conformation and bioactivity of the hormone (11, 22). Moreover, different experimental approaches have shown that the C-terminal part of TSHβ (deleted in the Q49X mutant) contains several domains required for high affinity TSH receptor binding and signal transduction (22, 25) (*i.e.* sequence 88–105

encompassing the seat belt region as well as sequence 58–69 within the β-hairpin βL3 loop). Therefore, mutations of the TSHβ gene represent an additional cause of discrepancy between immunoreactive and bioactive TSH levels commonly found in central hypothyroidism (26, 27).

The extremely high circulating values of the common α-GSU and their exaggerated response to TRH or L-T_4 withdrawal in the presence of low TSH, FSH, and LH represent a fundamental marker of ICH due to TSHβ gene mutations and indicate a very active, although deranged, synthetic process within thyrotropes (7, 11, 18). This is indirectly confirmed by the hyperplastic pituitary gland found by magnetic resonance imaging at the time of diagnosis and by the normalization of pituitary size during L-T_4 replacement therapy. These findings indicate that the assembly of Q49X TSHβ and the α-subunit itself is only partially conserved, so that a large amount of the synthesized α-subunit is circulating uncombined. Thus, the carboxyl-terminal region of TSHβ, lost in the Q49X mutant, appears to be required for optimal stabilization and maintenance of the heterodimer. Finally, it must be underlined that α-GSU has been found hypersecreted in the few cases of TSHβ gene mutations in which it was measured (Table 2) (11, 18). Moreover, the measurement of α-GSU is very useful in monitoring the adequacy of L-T_4 replacement therapy. In fact, TSH measurement is not a good parameter to follow-up L-T_4 replacement therapy in patients with any form of central hypothyroidism (28), and a decrease in α-GSU to an undetectable level was reported to occur in two patients with G29R mutations during replacement therapy with thyroid hormone (18). In the present case, L-T_4 undertreatment was revealed by the finding of elevated α-GSU values (Table 1), leading to the adjustment of the daily L-T_4 dose to a higher level (*i.e.* 3.0 μg/kg BW·day).

Finally, the dramatic consequences of delayed L-T_4 administration due to the missed neonatal diagnosis of congenital central hypothyroidism should promote further efforts aimed at improving neonatal screening programs to prevent cretinism in these cases. This might be achieved by the combined measurement of TSH and T_4 levels in the dry blood spot.

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