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Newborn Screening for Congenital Hypothyroidism

Edited by
Ernest M. Post and Natasha Heather

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About the Editors

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Ernest M. Post is a pediatric endocrinologist. He attended medical school, did his residency in pediatrics and fellowship in pediatric endocrinology at the SUNY Upstate Medical Center. He saw patients at Cooper University Hospital from 1983 to 2013 and now teaches at Cooper Medical School of Rowan University. He continues doing non-clinical work at the Hospital along with pursuing his other medical interests. He is co-Medical Director for Camp Nejeda for Children with Diabetes and involved in several newborn screening activities including chairing the NJ Newborn Screening Advisory Review Committee, the NJ Newborn Endocrine Advisory subcommittee, and the Clinical Laboratory Standards Institutes Committees Newborn Screening Expert Panel.

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Editorial

Congenital Hypothyroidism: Moving Ahead, but a Long Way Still to Go

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Newborn screening (NBS) for congenital hypothyroidism (CH) has been going on for more than fifty years, but we are still learning more about the process and the disease(s). This Special Issue of the *International Journal of Neonatal Screening* was conceived to focus on key contemporary issues surrounding CH NBS. These key issues include testing algorithms, CH NBS in low- and middle-income countries, premature infants, central hypothyroidism, and uncommon causes of CH.

We are very pleased to have met most of those goals. This Special Issue includes ten articles from Algeria, Australia, Brazil, Canada, Chile, China, Indonesia, Italy, Morocco, and New Zealand. The breadth of the information ranges from a report on the effects of not having CH NBS (Contribution 1) (lest we forget) to advocating for the expansion of CH NBS to include very rare causes (Contribution 2). The papers demonstrate both how far we have come (99.5% of newborns screened in one jurisdiction) and how much more we still have to accomplish (two thirds of patients experienced hypothyroidism after having been made euthyroid) (Contribution 3).

The papers included in this Special Issue touch on the high points of the advancements needed in CH NBS. While not explicitly part of the Special Issue, the most important goal is to increase universal CH NBS from, at present, approximately 30% worldwide coverage to 100% [1] (Contributions 4 and 5). Other major issues include providing good care to those with CH (Contribution 3), reviewing screening cutoffs (Contribution 6), finding the best protocol for re-screening premature babies (Contributions 7 and 8), identifying the causes of the rise in CH (Contribution 9), and discovering the best use of genetic information in CH NBS (Contribution 10).

We look forward to future publications on the efforts undertaken to address these areas.

Conflicts of Interest: The authors declare no conflicts of interest.

List of Contributions

1. Djermane, A.; Ouarezki, Y.; Boulesnane, K.; Kherra, S.; Bouferoua, F.; Bessahraoui, M.; Selim, N.; Djahlat, L.; Mohammedi, K.; Bouziane Nedjadi, K.; et al. The Burden of Congenital Hypothyroidism Without Newborn Screening: Clinical and Cognitive Findings from a Multicenter Study in Algeria. *Int. J. Neonatal Screen.* **2025**, *11*, 78. https://doi.org/10.3390/ijns11030078.
2. Olivieri, A.; Vigone, M.C.; Salerno, M.; Persani, L. Is It Time to Expand Newborn Screening for Congenital Hypothyroidism to Other Rare Thyroid Diseases? *Int. J. Neonatal Screen.* **2025**, *11*, 65. https://doi.org/10.3390/ijns11030065.

3. Sosova, I.; Archibald, A.; Rosolowsky, E.W.; Rathwell, S.; Christian, S.; Rosolowsky, E.T. Evaluation of the First Three Years of Treatment of Children with Congenital Hypothyroidism Identified through the Alberta Newborn Screening Program. *Int. J. Neonatal Screen.* **2024**, *10*, 35. https://doi.org/10.3390/ijns10020035.
4. Pulungan, A.B.; Puteri, H.A.; Faizi, M.; Hofman, P.L.; Utari, A.; Chanoine, J.-P. Experiences and Challenges with Congenital Hypothyroidism Newborn Screening in Indonesia: A National Cross-Sectional Survey. *Int. J. Neonatal Screen.* **2024**, *10*, 8. https://doi.org/10.3390/ijns10010008.
5. Wahoud, F.; Essadki, S.; Zirar, K.; Lamsyah, R.; Hajjaji, W.; Amrani, R. Implementation of Neonatal Screening Program for Congenital Hypothyroidism in Eastern Morocco. *Int. J. Neonatal Screen.* **2025**, *11*, 55. https://doi.org/10.3390/ijns11030055.
6. Teixeira Palla Braga, N.; Vilela Antunes, J.M.; Colosimo, E.A.; Alves Dias, V.M.; Januário, J.N.; Novato Silva, I. Impact of Lowering TSH Cut-Off on Neonatal Screening for Congenital Hypothyroidism in Minas Gerais, Brazil. *Int. J. Neonatal Screen.* **2024**, *10*, 52. https://doi.org/10.3390/ijns10030052.
7. Greaves, R.F.; Northfield, J.-A.; Cross, L.; Mawad, N.; Nguyen, T.; Tan, M.; O'Connell, M.A.; Pitt, J. Managing Newborn Screening Repeat Collections for Sick and Preterm Neonates. *Int. J. Neonatal Screen.* **2024**, *10*, 63. https://doi.org/10.3390/ijns10030063.
8. Brown, A.; Hofman, P.; Webster, D.; Heather, N. Screening Blind Spot: Missing Preterm Infants in the Detection of Congenital Hypothyroidism. *Int. J. Neonatal Screen.* **2025**, *11*, 37. https://doi.org/10.3390/ijns11020037.
9. Grob, F.; Cavada, G.; Lobo, G.; Valdebenito, S.; Perez, M.V.; Donoso, G. Incidence of Congenital Hypothyroidism Is Increasing in Chile. *Int. J. Neonatal Screen.* **2025**, *11*, 58. https://doi.org/10.3390/ijns11030058.
10. Ye, L.; Zhang, Y.; Feng, J.; Huang, C.; Wang, X.; Han, L.; Huang, Y.; Zou, H.; Zhu, B.; Miao, J. Newborn Genetic Screening Improves the Screening Efficiency for Congenital Hypothyroidism: A Prospective Multicenter Study in China. *Int. J. Neonatal Screen.* **2024**, *10*, 78. https://doi.org/10.3390/ijns10040078.

Reference

1. Arrigoni, M.; Zwaveling-Soonawala, N.; LaFranchi, S.H.; van Trotsenburg, A.S.P.; Mooij, C.F. Newborn screening for congenital hypothyroidism: Worldwide coverage 50 years after its start. *Eur. Thyroid. J.* **2025**, *14*, e240327. [CrossRef] [PubMed]

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Article

The Burden of Congenital Hypothyroidism Without Newborn Screening: Clinical and Cognitive Findings from a Multicenter Study in Algeria

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Abstract

The absence of biochemical newborn screening (NBS) delays the diagnosis and treatment of congenital hypothyroidism (CH), resulting in irreversible neurodevelopmental damage. To determine the age at diagnosis for CH among Algerian children and to describe its clinical and biological characteristics, etiology, and outcome, we conducted a multicenter retrospective cohort study involving 288 children with CH across 20 pediatric centers between 2005 and 2023. The median age at diagnosis was 1.6 months, and only 28% of patients started treatment before 30 days. Prolonged neonatal jaundice was the most frequently presented symptom (58%), severe CH (fT_4 < 5 pmol/L) was observed in 35% and 52% received an insufficient initial dose of L-T_4. The median IQ of the 47 patients tested was 86; 11% had an IQ < 70, and a negative correlation was found between age at diagnosis and IQ ($r = -0.48$, $p = 0.001$). In children reassessed at age 3, 51% had normal thyroid function, indicating transient CH. Delayed diagnosis and suboptimal treatment of CH remain major challenges in Algeria, leading to substantial neurodevelopmental deficits. Pediatricians must remain cognizant of early clinical signs of CH to allow for timely diagnosis and intervention. Biochemical NBS for CH in Algeria is needed.

Keywords: congenital hypothyroidism; newborn screening; neurodevelopmental; IQ; treatment; Algeria

1. Introduction

Congenital hypothyroidism (CH) is one of the most common causes of preventable intellectual disability in children. In countries where universal newborn screening (NBS) programs are established, CH is routinely detected within the first two weeks of life—allowing for early initiation of levothyroxine therapy and preventing long-term neurodevelopmental impairments [1]. However, in the absence of screening, diagnosis often relies on clinical suspicion alone, which may be delayed due to the subtle or nonspecific nature of early symptoms [2].

In Algeria, there is currently no nationwide NBS program for CH. As a result, the diagnosis is frequently based on the recognition of clinical signs, such as prolonged neonatal jaundice, constipation, hypotonia, or delayed growth—signs that often appear after the critical period for optimal neurological development. This delayed recognition contributes to late initiation of treatment and increases the risk of permanent psychomotor and intellectual deficits [3].

In this context, our study aims to evaluate, for the first time on a national scale, the age at diagnosis of CH in Algeria. It also seeks to characterize the clinical and biological features at presentation, identify etiological patterns, assess the adequacy of treatment, and investigate the neurodevelopmental consequences of delayed diagnosis. Our ultimate goal is to provide evidence to support the implementation of a universal NBS program and to raise awareness among healthcare professionals and decision makers about the importance of the early detection of CH.

2. Methods

This was a multicenter observational cohort study.

2.1. Objectives

The primary outcome was to determine the age at diagnosis of patients with CH in Algeria, where newborn screening is not established.

The secondary outcomes were to determine the clinical and biological characteristics at presentation, the etiology of CH, and the neurodevelopmental outcome, as well as to explore the correlation between neurodevelopmental outcomes and age at diagnosis.

2.2. Study Design and Population

We retrospectively reviewed the medical records of 432 children followed for CH diagnosed between February 2005 and September 2023 across twenty pediatric departments in Algeria. Neurodevelopmental assessments were conducted prospectively in a subset of patients as part of standardized follow-up evaluations.

The study population included all children with CH aged 0 and 18 years who attended an outpatient clinic from January 2017 to December 2023.

The exclusion criteria were central hypothyroidism, Down syndrome, patients who were not treated, and those who experienced early discontinuation of treatment or were lost to follow-up.

2.3. Data Collection

The following data were collected for analysis: reason for referral, clinical characteristics at diagnosis, auxological data, serum TSH and fT_4, radiological evaluation (knee X-rays),

etiology based on imaging, and dose of L-T4 treatment. TSH values above 100 mU/L (not reported precisely) were included in the analysis by assigning them a value of 100 mU/L. Treatment under the recommended dose was considered inadequate [4,5].

2.4. Definitions

Primary hypothyroidism was defined by elevated serum TSH (age-adjusted, minimal TSH > 8 mU/L) and/or a low fT_4 (<10 pmol/L). Due to insufficient follow-up data, subclinical hypothyroidism could not be reliably identified or analyzed.

Two etiological groups of CH were defined based on the results of thyroid ultrasound (US) and/or pertechnetate scintigraphy: thyroid dysgenesis (TD), including (athyreosis, ectopic gland, and orthotopic hypoplasia) and gland in situ (GIS) of a normal or increased size (including all patients with normal scintigraphy/US and patients with a goiter detected clinically). Cases with GIS were considered suggestive of dyshormonogenesis (DH).

The initial L-T_4 dose was considered insufficient when it was below 9 μg/kg/day in the first 3 months, and below 6 μg/kg/day after 3 months.

2.5. Neurodevelopmental Assessment

The neurodevelopmental status of the children was determined using several measures, including psychomotor delay (defined as a delay in attaining developmental milestones) and school progression (absence of schooling, grade repetition, or poor school performance) for the school-aged children (defined as 6–18 years). In addition, IQ was measured in some patients using the Arabic version of the Wechsler Intelligence Scale for Children—IV for children aged 6 to 16 years; children under 6 years of age were assessed using age-appropriate standardized tools such as the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III), the Columbia Mental Maturity Scale, and the Kohs Block Design Test [6,7]. Scores of IQ < 70 are considered to indicate intellectual disability, and ≥85 are considered normal intelligence. We analyzed and compared the characteristics between low IQ (<85) and normal IQ (≥85) groups.

2.6. Reassessment

In some children who did not undergo scintigraphy before treatment, treatment was stopped after 3 years, and serum TSH and fT_4 were reassessed. CH was then classified as either transient if TSH did not rise after treatment withdrawal, and as permanent otherwise [8].

2.7. Ethics

An ethics statement is not applicable because this study is based exclusively on a clinical audit.

2.8. Statistical Analysis

The data were expressed as mean ± SD or median (range) as appropriate. For comparisons between two groups, independent-samples *t*-tests, Mann–Whitney or Chi-square (χ^2) tests were used. For more than two groups, one-way analysis of variance (ANOVA) was used. Correlational analyses were performed using Pearson's correlation coefficients. To identify risk factors for low IQ and psychomotor delay, multivariate analyses such as linear regression, logistic regression, and multiple regression models were applied. Receiver Operating Characteristic (ROC) analysis was used to evaluate the performance of a diagnostic test. Epi Info 7, Excel, and Medcalc were used to collect and analyze the data.

3. Results

Of 432 CH patients, 288 (66.6%) were included in the study, with 144 excluded for reasons such as missing data, central hypothyroidism, Down syndrome, or lack of data on treatment/follow-up. Figure 1 provides a visual representation of the patient selection and exclusion process, leading to the final group of 288 patients whose clinical characteristics were analyzed in the study.

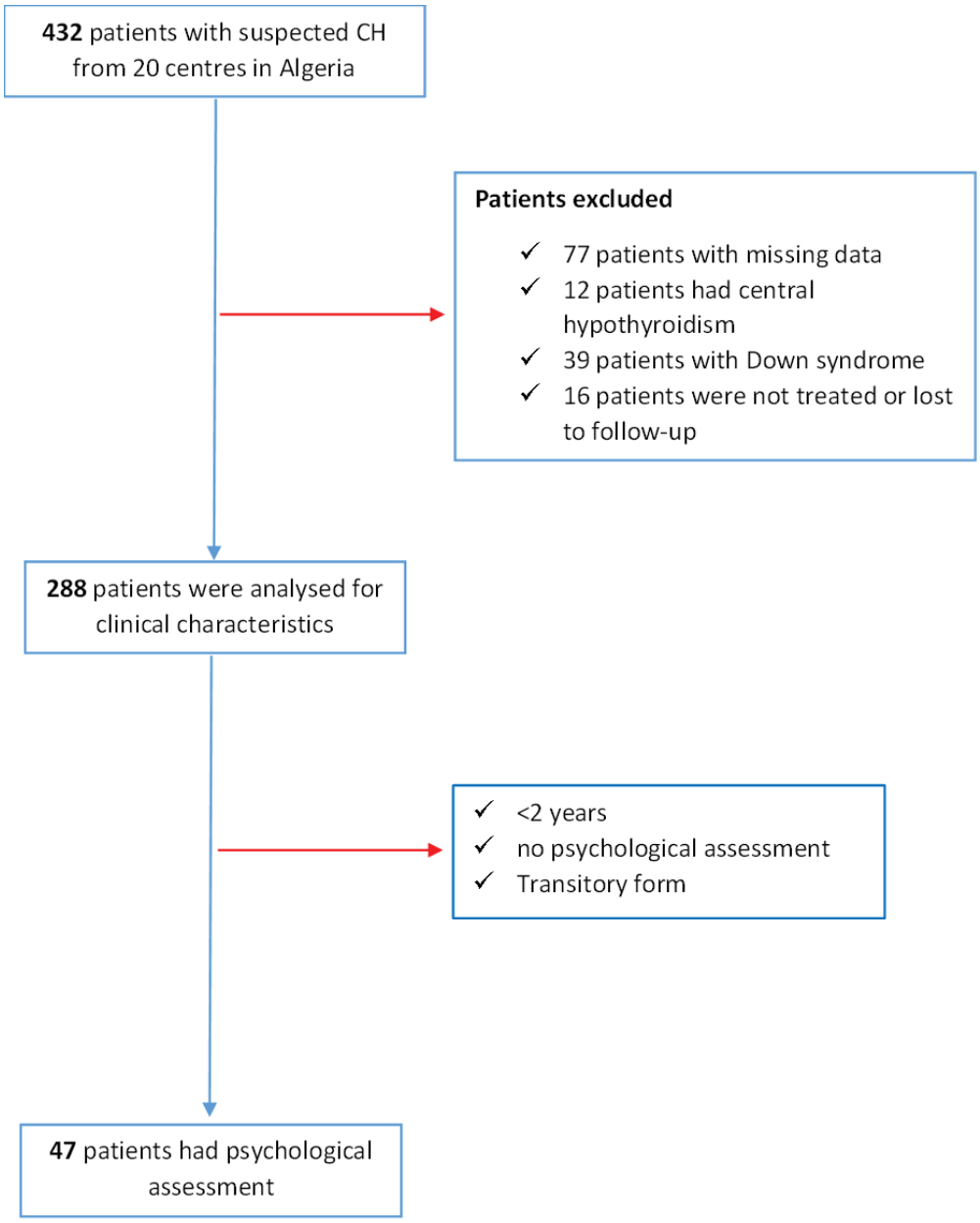

Figure 1. Flowchart of the study.

3.1. Characteristics of the CH Population

Age at diagnosis: The median age at diagnosis was 1.6 (0.05–150) months, but the median age at the initiation of treatment was 2 months, ranging between <1 and 156 months. Less than half of the patients (35%) were diagnosed before 1 month, while more than 37% of the patients were diagnosed after three months, and 18% after 1 year of age (Figure 2). The oldest CH case was diagnosed at 150 months, presenting with short stature and developmental delay.

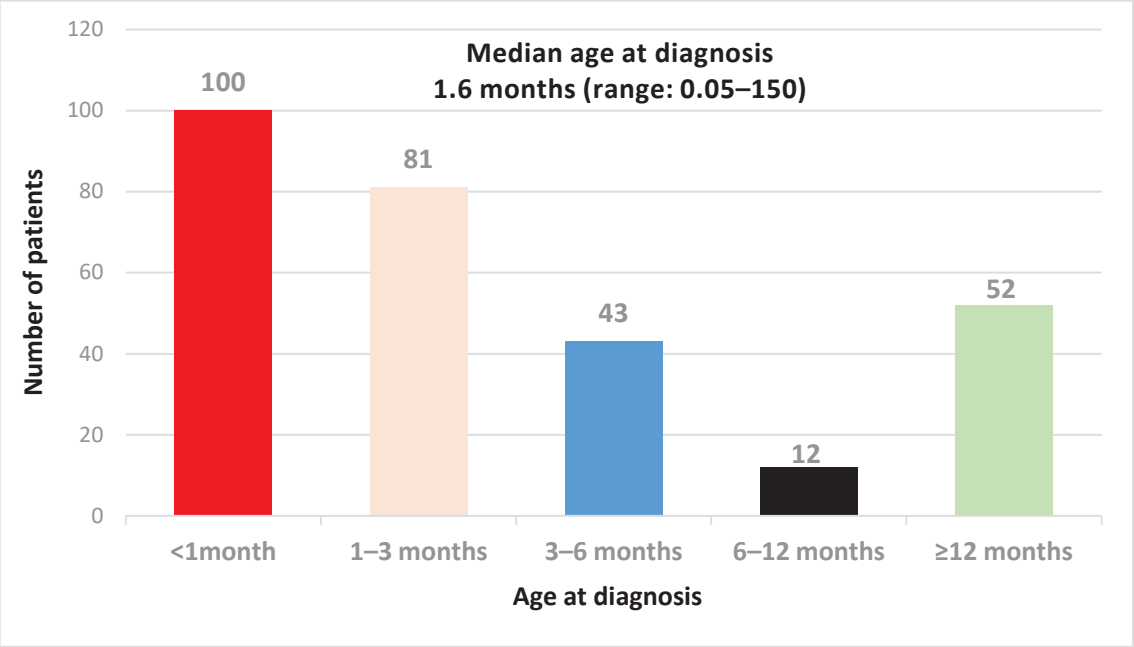

Figure 2. Age at diagnosis in months.

Sex ratio: There were 159 females (55%) and 129 males (45%), leading to a female-to-male ratio of 1.23.

Consanguinity and family history: Consanguinity was reported in 75 patients (26%), a family history of thyroid dysfunction was noted in 105 patients (36%), and CH was reported in 27 (9%) siblings from 12 families. Moreover, an extrathyroidal congenital abnormality was present in 44 patients, with cardiac defects being the most common (8%) (Table 1).

Table 1. Family history, etiological groups, and comorbidities.

Family History		
Consanguinity	*n*, (%)	75 (26%)
Family history of CH in a sibling	*n*, (%)	27 (9%)
Etiology		
Dysgenesis	*n*, (%)	109 (38%)
Athyreosis	*n*, (%)	52 (18%)
Ectopy	*n*, (%)	17 (6%)
Hypoplasia	*n*, (%)	40 (14%)
Gland in situ	*n*, (%)	150 (52%)
Goiter	*n*, (%)	25 (9%)
Undetermined	*n*, (%)	29 (10%)
Associated abnormalities	*n*, (%)	44 (15%)
Heart defects	*n*, (%)	23 (8%)
Renal defects	*n*, (%)	9 (3%)

Birth characteristics: The average gestational age was 38 weeks, and prematurity was noted in 27 patients (9%), while 11% of the patients were born SGA. The mean birth weight, length, and head circumference were 3.2 kg, 49.6 cm, and 34.4 cm, respectively (Table S1).

Cause of referral and signs at presentation (Figure 3): The most common symptom leading to the suspicion of CH and referral was jaundice for more than 10 days (36.5%), whereas it was clinically observed at diagnosis in 58% (Figure S1). Other symptoms included

constipation, psychomotor delay, short stature, hypotonia, and goiter. Only 13% had a targeted screening with serum TSH during the neonatal period because of a family history of hypothyroidism (sibling with CH or mother with hypothyroidism).

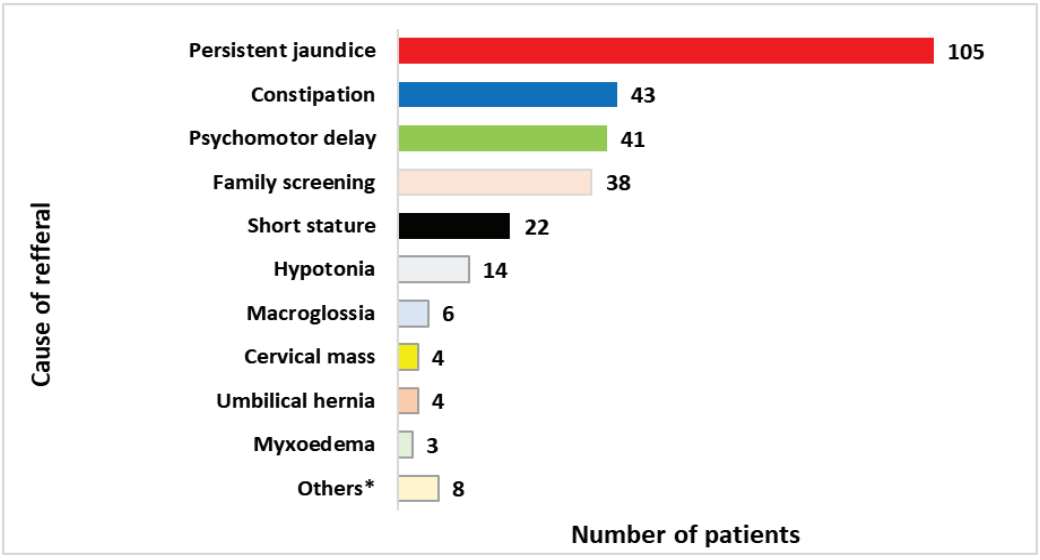

Figure 3. Cause of referral. * No CH-associated clinical signs or not reported.

Forty-two (15%) patients had short stature (Height < −2 SD) at diagnosis, and seventeen (6%) had a BMI > +2 SD. The reason for referral had a significant impact on the age at diagnosis: jaundice and constipation were common before 3 months, while psychomotor delay and short stature were reported in patients diagnosed after 12 months (Table S2). Those who presented with prolonged jaundice at a median age of one month (range: 0.05–34) or were screened at a median age of 0.3 months (range: 0.07–12) had the youngest age at diagnosis, whereas those who presented with short stature had the oldest age at diagnosis (median 39 months (range: 2.33–150) (Figure 4).

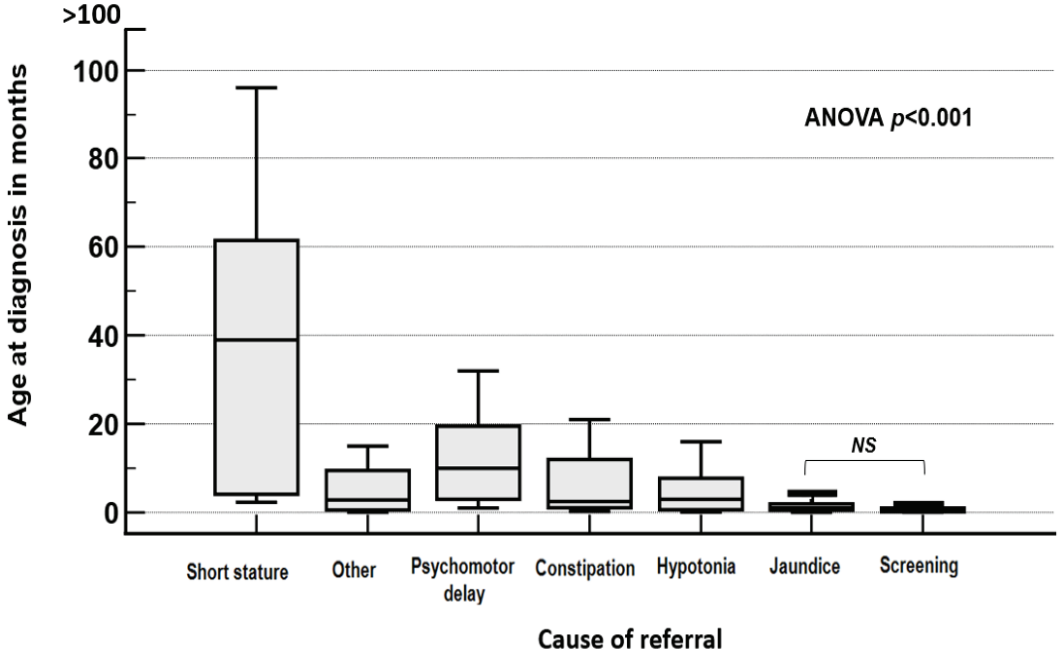

Figure 4. Age at diagnosis in months according to the cause of referral. NS: No significant difference. Boxplot: middle line = median, box = interquartile range, whiskers = range.

Biological data (Table 2): The diagnosis of hypothyroidism was confirmed through the measurement of serum TSH and fT_4 prior to treatment in all patients. The median TSH value before treatment was 65.35 mU/L. Mean ± SD fT4 levels were 7.6 ± 6.2 pmol/L (range 0.01–20). A total of 43% were found to have extremely high TSH levels (>100 mU/L), 56% had low fT_4 levels (<10 pmol/L), and 34% had fT_4 levels <5 pmol/L, indicating severe CH.

Table 2. Biology of CH patients at diagnosis.

Biological Data		
TSH mU/L *	Median (range)	65.35 (8.12–>100)
fT4 pmol/L	Mean ± SDS (range)	7.6 ± 6.2 (0.01–27)
TSH >100 mU/L	*n*, %	125 (43.4%)
TSH 40–100 mU/L	*n*, %	54 (18.7%)
TSH 20–40 mU/L	*n*, %	32 (11.1%)
TSH < 20 mU/L	*n*, %	77 (26.7%)
fT4 < 5 pmol/L	*n*, %	102 (35.4%)

* TSH values >100 mU/L were recorded at 100 mU/L.

3.2. Imaging Exams and Aetiological Groups

Knee radiography was performed in 97 patients (34%): no ossification center was shown in 34 full-term infants, indicating a prenatal onset of hypothyroidism. Thyroid US was performed in 251 patients (87%), and pertechnetate scintigraphy in 137 (48%). The GIS group was the largest etiological group (150 cases, 52%), with goiters accounting for only 9% of the cases, while thyroid dysgenesis was found in 109 cases (38%). In 29 (10%) cases, the etiological group could not be defined because of the lack of an imaging study. The dysgenesis group showed significantly more severe forms, while consanguinity and female sex were more frequent in the GIS group (Table 3).

Table 3. A comparison of the clinical and biological data according to the etiological group.

	Global CH Cohort ***N* = 288**	**GIS/Goiter** ***N* = 150**	**Ectopy/Hypoplasia** ***N* = 57**	**Athyreosis** ***N* = 52**	**Undetermined** ***N* = 29**	***p*-Value**
Age at diagnosis (months) Median (range)	2 (0.1–375)	1.5 (0.05–51)	4 (0.07–155)	2 (0.06–150)	0.86 (0.13–32)	0.001 *
M/F Sex ratio	129/159 0.81	80/69 1.2	24/33 0.7	10/42 0.24	15/15 1	0.0003
Consanguinity% (*N*)	26% (75)	31% (47)	23% (13)	13.5% (7)	27% (8)	0.078
TSH mU/L Median (range)	65.3 (8.1–>100)	50 (8.1–>100)	96.3 (10.1–>100)	100 (17.9–>100)	47.5 (10–>100)	0.034 *
TSH > 100 mU/L% (*N*)	43% (125)	35% (53)	39% (22)	73% (38)	41% (12)	<0.0001
fT_4 (pmol/L) Mean ± SD (range)	7.6 ± 6.4 (0.01–25.4)	8.8 ± 6.1 (0.01–25.7)	7.2 ± 5.5 (0.02–27)	2.6 ± 2.7 (0.01–10.6)	8.8 ± 2.3 (0–25.4)	0.003
fT_4 < 5 pmol/L %, (*N*)	35% (102)	26% (39)	37% (21)	60% (31)	38% (11)	0.0002

* Comparison between in situ gland (GIS)/dysgenesis (all data).

3.3. Treatment

Levothyroxine (L-T_4) treatment was initiated at a median age of 2 months, with a range between 0.06 (2 days) and 150 months. Only 82 (28%) of the patients were started on L-T_4 before 30 days of age. Among the 288 patients included, 228 (79%) began L-T4 treatment within 15 days following the biological diagnosis of hypothyroidism, while 42

(15%) patients began treatment more than one month after diagnosis. The mean starting dose of L-T_4 was 6.9 ± 4.1 (range 0.6–25.6 µg/kg/day). Patients aged less than 1 month at diagnosis received a higher dose (7.8 ± 3.7 µg/kg/day) (Table S3). One hundred forty-nine (52%) received an insufficient initial L-T_4 dose, with 51% receiving less than 9 µg/kg/day in the first 3 months and 53% receiving less than 6 µg/kg/day after 3 months (Figure 5).

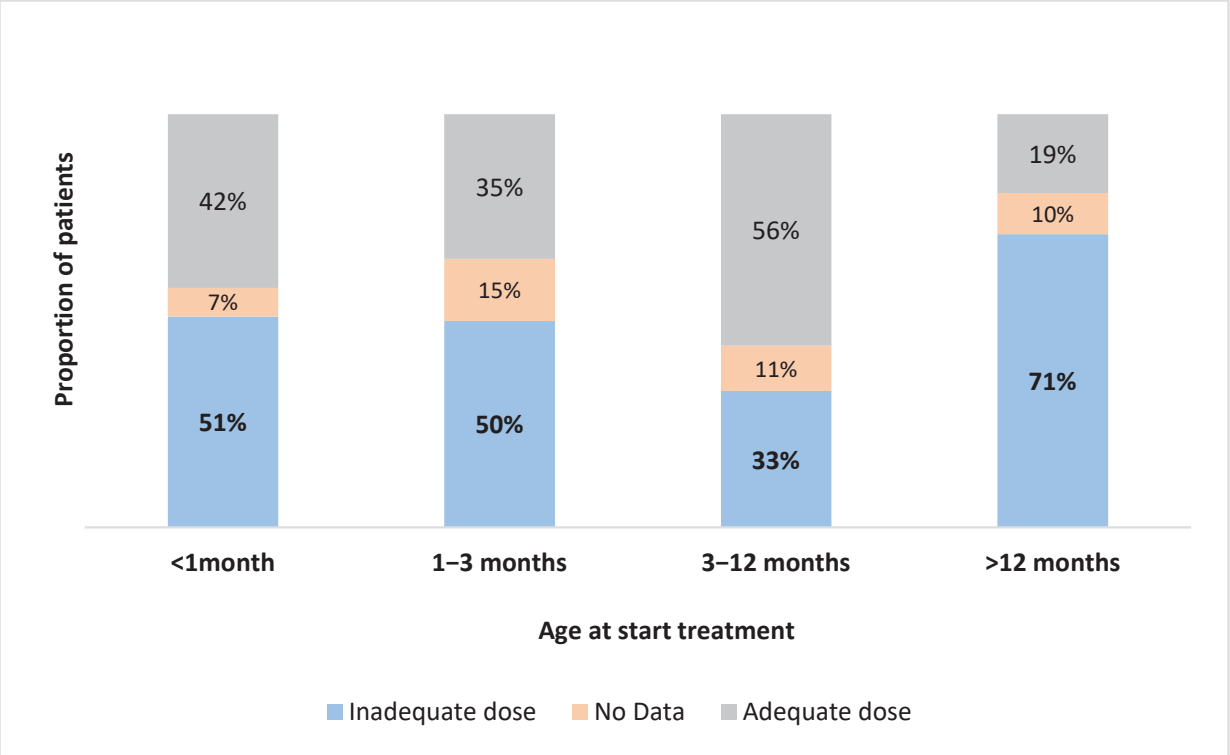

Figure 5. Age at LT-4 treatment at initiation.

3.4. Outcomes

3.4.1. Transient vs. Permanent CH

Forty-seven patients (16%) underwent biological reassessment after stopping treatment, of which twenty-four (51%) had normal thyroid function, indicating transient hypothyroidism (Table 4).

Table 4. A comparison of clinical and biological data between permanent and transient CH in 47 patients who underwent a clinical reassessment at age 3.

	Transient CH *N* = 24	**Permanent CH *N* = 23**	***p***
Sex ratio M/F	12/12	8/15	0.297
Age at diagnosis in months—median (range)	4.3 (0.16–46)	2.4 (0.1–12)	0.440
TSH mU/L—median (range)	15.98 (4.3–>100)	100 (7.6–>100)	0.002
TSH > 100 mU/L, *n*, (%)	3 (12.5%)	11 (48%)	0.009
TSH < 10 mU/L, *n*, (%)	4 (17%)	0 (0%)	0.037
fT_4 pmol/L—mean (range)	13.4 (0.1–17.2)	7.5 (0.1–18.1)	0.003
fT_4 < 10 pmol/L *n*, (%)	5 (21%)	14 (61%)	0.011
Gland in situ *n*, (%)	23 (96%)	10 (43%)	0.001
Dysgenesis *n*, (%)	1 (5%)	13 (57%)	

In patients with transient CH, initial TSH levels were significantly lower and fT_4 was significantly higher at diagnosis than in those with permanent CH. The transient CH cases were more frequent in the GIS group (Table 4).

3.4.2. Neurodevelopmental Assessment (Table 5)

A total of 16 patients (6%) were reported to have a language delay, 5 (2%) had hearing loss, while 43 (15%) were described as having a psychomotor delay.

School progression data were available for 88 of 206 school-aged children (43%): 27 (31%) had repeated a grade or had left regular school.

A high rate of delayed treatment beyond the first month of life was observed among patients with neurodevelopmental impairments. This was particularly pronounced in extreme cases, including psychomotor delay (97.7%), language delay (87.5%), school failure (77.8%), and IQ below 85 (>85%).

Among children diagnosed at age 12 months or older, 6/52 (11.5%) were found to have a severe developmental delay. The ROC plot analysis showed that the threshold line for an age of 1.94 months is associated with psychomotor delay with a sensitivity of 87.2%, a specificity of 66.7%, and an AUC of 0.815, $p < 0.001$ (Figure 6). Among the one hundred and fifty (151) children older than 1.94 months at diagnosis, sixty (40%) were labeled with psychomotor delay, nine (6%) of whom had severe CH. Using multiple regression, children with CH diagnosed after 2 months of age had an OR of 7.77 [2.88–20.67] of having psychological delay, while this OR was 0.29 [0.07–1.18] in those diagnosed before 1 month of age (Table S4).

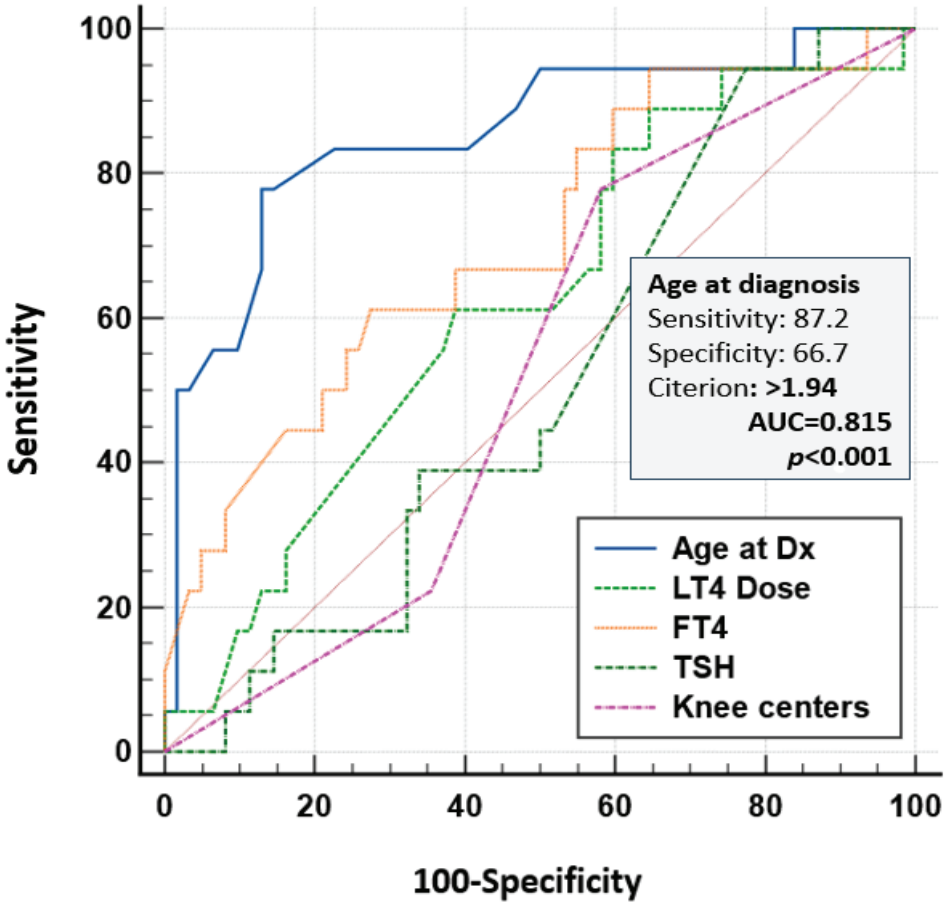

Figure 6. ROC comparison of risk factors for intellectual disability. ROC: Receiver Operating Characteristic (ROC); LT4: Levothyroxine.

Table 5. Neurodevelopmental data.

Neurodevelopmental Data	*N*, (%)	Age at Evaluation Mean ± SD	Age Treatment Median	Treatment *n*, %	
				≤1 Month	≥3 Months
Psychomotor delay	43 (15%)	23.3 ± 25.1 months	13.9 months	1/43 (2%)	36/43 (84%)
Language delay	16 (6%)	18.4 ± 18.1 months	3.6 months	2/16 (12.5%)	9/16 (56%)
School-aged children	88/206 (43%)	11.6 ± 3.4 years	3 months	20/88 (23%)	47/88 (53%)
School failure	27/88 (31%)	10.8 ± 3.9 years	4 months	6/27 (22%)	16/27 (59%)
IQ assessment *N* = 47					
Normal IQ ≥ 85	29 (62%)	5.1 ± 1.7 years	1.1 months	13/29 (45%)	9/29 (31%)
Low IQ < 85	13 (28%)	5.2 ± 1.8 years	3 months	2/13 (15%)	8/13 (61.5%)
Very low IQ < 70	5 (11%)	4.8 ± 2.5 years	29 months	0/5 (0%)	4/5 (80%)
Cognitive disharmony	7 (15%)	5.6 ± 1.3 years	2 months	2/7 (28.6%)	3/7 (43%)

3.4.3. IQ Evaluation

Out of 47 (16%) patients with an IQ evaluation (Table 5), only 62% had a normal value, while 11% had an IQ < 70, the WHO definition of intellectual disability (Figure 7). The median age at IQ assessment was 5 years (range: 2.9–9). The mean IQ was 86.1 ± 15.2 (50–112). This result was significantly lower than the theoretical mean IQ of 100 in the general population ($p < 0.0001$) (Figure 7). Among the children who underwent IQ testing, only three were later classified as having transient congenital hypothyroidism, and all were within the normal range (93, 100, and 109). Thirty-seven of the forty-seven IQ assessments (79%) were performed at the same center with standardized methods, minimizing inter-center variability and improving data consistency.

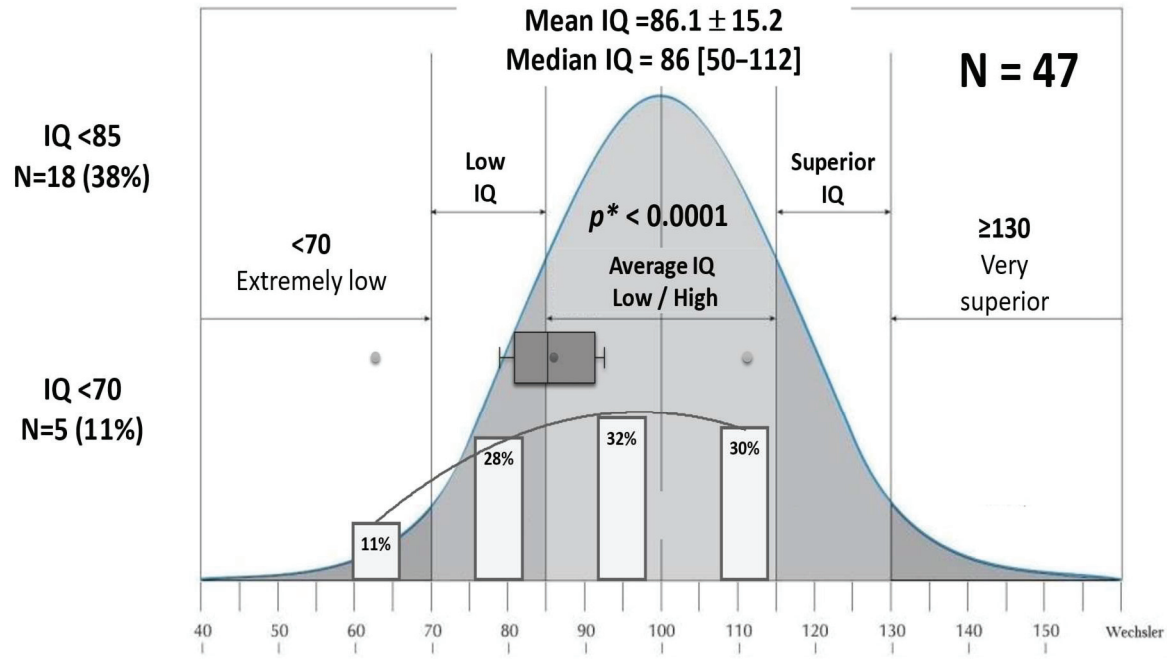

Figure 7. The results of the IQ assessment in 47 patients. * Comparison with the theoretical mean.

Children with IQ < 85 (n = 18) did not differ from those with IQ ≥ 85 (n = 29) in terms of gender, age at IQ test, and etiology (Table 6).

Table 6. Comparison of IQ groups.

		Global ***N* = 47**	**IQ < 85** ***N* = 18**	**IQ ≥ 85** ***N* = 29**	***p***
Sex M/F		15/32	5/13	10/19	0.63
Age IQ test years—mean (range)		5.3 ± 1.8 (2.5–9.3)	5.5 ± 2.1 (3–9.3)	5.2 ± 1.7 (2.5–8)	0.68
Age at treatment in months—median (range)		2 (0.1–69)	3.75 (0.2–69)	1.1(0.1–42.7)	0.01
Age start L-T_4	≤1 month (*n*,%)	16 (25.5%)	3 (17%)	13 (45%)	0.05
	>1 month (*n*, %)	31 (74.5%)	15 (83%)	16 (55%)	
Etiology	Dysgenesis	25 (53%)	13 (72%)	12 (41%)	0.05
	Gland in situ	21 (45%)	5 (28%)	16 (55%)	
L-T_4 Dose μg/kg/day	<9 μg/kg/day	27 (54%)	12 (67%)	15 (52%)	0.26

At the start of treatment, children with IQ < 85 were significantly older (median 3.75 months, range: 0.2–69) than children with IQ ≥ 85 (median 1.1 months, range: 0.1–42.7). The IQ ≥ 85 group had started treatment before one month (45%), and with a dose higher than 9 μg/kg/day than those with IQ < 85 (38% vs. 22%). Seven (15%) patients with a normal IQ have a cognitive disharmony (Table 6).

Our analysis shows a significant negative correlation between IQ and age at diagnosis (r = −0.48, $p = 0.001$) (Figure 8).

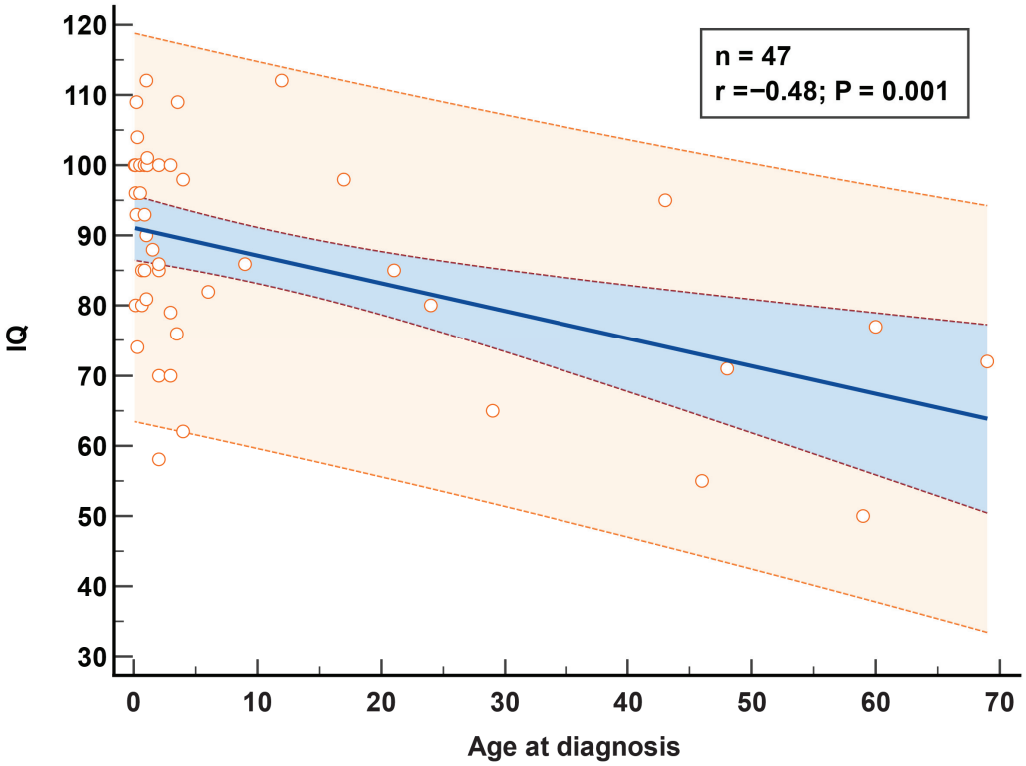

Figure 8. Correlation between age at diagnosis and IQ. Blue: 95% confidence interval; brown shading: 95% prediction interval.

Logistic regression indicates that dysgenesis significantly increases the risk of low IQ (OR = 5.55 [1.1–25.7]). A second IQ test on 11 CH patients at a median age of 8 years (range: 4.5–10) showed improvement to an IQ ≥ 85 in five cases (45.5%).

4. Discussion

In the absence of a standard biochemical NBS program, the diagnosis of CH relies on a systematic and rigorous clinical examination of newborns at birth and during the first weeks of life. Despite this, not all patients are diagnosed in time to avoid intellectual disability. Even though the inclusion period spans more than 15 years, most pediatric endocrinology centers participating in this study have existed for less than 10 years; so, despite an estimated 10-year recruitment period, the recruitment concerned only some hospital-based pediatric clinics, and therefore does not allow for an estimation of the population prevalence of CH. However, published studies indicate that the incidence of CH ranges from 1 in 7000 to 1 in 10,000 births in populations without screening, while this rate increases to between 1 in 1100 and 1 in 3000 births when screening is implemented, depending on the TSH threshold used [9–12].

Clinical Diagnostic Challenges: The difficulty in early clinical diagnosis stems from frequently subtle or absent symptoms at birth [9,13]. In our study, jaundice emerged as the primary diagnostic clue, with a median age at presentation of 1.66 months. This aligns with established knowledge that CH often manifests as prolonged neonatal jaundice [2,13,14] accompanied by other characteristic symptoms such as constipation, macroglossia, delayed fontanel closure, umbilical hernia, and coarse facial features [14–16]. The clinical diagnosis remains particularly challenging, as most affected infants exhibit highly nonspecific symptoms, with only about 5% of CH cases presenting sufficiently distinctive signs within the first few days of life to allow for a prompt diagnosis [17]. Furthermore, it is concerning that approximately 5–10% of newborns with CH are not detected by primary screening programs, irrespective of whether the screening targets thyroxine (T_4) or thyroid-stimulating hormone (TSH) [18].

Delayed diagnosis of CH remains alarmingly common in many parts of the world, when NBS programs are inadequate or absent, showing persistent gaps in early detection [19]. In regions lacking widespread NBS, the median age at diagnosis is significantly delayed, often exceeding 45 days, which contributes to poorer developmental outcomes (Table 7) [3,15,16,20–29]. Aligning with global data, our results demonstrate a concerning delay in CH diagnosis, with a mean age at diagnosis of 2 months, and 65% of cases identified beyond the neonatal period (>1 month).

Table 7. Original studies about age at diagnosis in unscreened CH patients.

Reference (Original Study)	Country	Period	Diagnosed in Neonatal Period	Late Diagnosis Rates	Neurodevelopmental Outcome
Raiti 1971 [15]	UK	–	6% < 1st month 22% < 3 months	16% < 6 months 55% by 2 years	IQ < 90 (50%) 53% treatment > 6 months
De Jonge 1976 [20]	Netherlands	1972–1974	10% <1 month	50% at 3 months	34% IQ > 90, 17% IQ < 50
Alm 1978 [3,16]	Sweden	1969–1975	20%	52% after 3 months	41% IQ < 85 and/or neurological abnormalities
Wolter 1979 [21]	Belgium	–	7% < 1st month 46% < 3 months	21% > 1 year	IQ < 80 (23%); Normal IQ if treated <3 months
Jacobsen 1981 [22]	Denmark	1970–1975	10% 1st month	70% by 1 year	46% intellectual disability
Tarim 1992 [23]	Turkey	1964–1989	3.1%	55.4% after 2 years	21.4% inability to speak 18.1% inability to walk
Nasheiti 2005 [24]	Iraq	1993–2003	25%	75% beyond neonatal period	47.5% intellectual disability

Table 7. *Cont.*

Reference (Original Study)	Country	Period	Diagnosed in Neonatal Period	Late Diagnosis Rates	Neurodevelopmental Outcome
Chen 2013 [25]	South Asian countries	1997–2008	Taiwan 56% <3 months	22% > 1 years Pakistan, India 70% >1 year	Diagnosis >3 months, higher risk of developmental delay (HR = 1.97)
Niang 2016 [26]	Senegal	2001–2014	7%	78.5% >6 months	73% intellectual disability 2.5% at school
Deliana 2016 [27]	Indonesia	1992–2002	Minimal	53% at 1–5 years 6.7% after 12 years	62.5% intellectual disability
Saoud 2019 [28]	Syria	2008–2012	>25% 1st month	75% beyond neonatal period	37.1% psychomotor delay 81% diagnosis >6 months
Kahssay 2025 [29]	Kenya *	2015–2020	5% < 1st month	80% 6–11 months 15% > 1–2 years	60% developmental delay
Our study	Algeria	2005–2023	35% < 1st month	65% ≥1 month	28% IQ < 85, 11% IQ < 70

* Facility-based study, HR: Hazard ratio.

4.1. The Consequences of Delayed Diagnosis and Treatment

The effects of delayed diagnosis and treatment of CH are often severe and irreversible, significantly impacting both development and quality of life [15]. Early studies indicate that delaying treatment beyond three months of age, and even more beyond the age of six months, causes irreversible intellectual disability with a higher risk of lower IQ scores and neuropsychological deficits, collectively referred to as cretinism [18,30–32]. Late initiation of L-T_4 therapy has been shown to negatively affect cognitive functioning and overall well-being [33]. Research by Leger et al. provides strong evidence that, during early adolescence, there is a clear link between disease severity at the time of diagnosis, the adequacy of treatment during follow-up, and poor school performance among individuals treated for CH from the neonatal stage [34,35]. Several factors have been identified as predictors of worse intellectual outcomes in children with CH, including initial serum T_4 levels at diagnosis, the timing of treatment initiation, prolonged time to normalize thyroid hormone levels, maternal education, socioeconomic status, and the frequency of clinic visits during the first year of life [5,31,32,36,37]. Cognitive problems may persist despite treatment, including difficulties with visual–spatial abilities, language development, fine motor skills, memory, and attention [38]. Beyond the cognitive domain, untreated cases often exhibit severe growth impairments, including stunted growth and delayed bone maturation [39]. Neurological complications such as spasticity, gait abnormalities, dysarthria, mutism, and behavioral disorders may also develop in affected individuals when treatment is delayed [33].

4.2. Etiological Diagnosis and Genetic Factors in Congenital Hypothyroidism

CH is predominantly accounted for by two main etiological categories: thyroid dysgenesis (TD) and dyshormonogenesis (DH). TD represents approximately 80–85% of CH cases and includes conditions such as ectopy, athyreosis, and orthopic hypoplasia, which are rarely linked to mutations in transcription factor genes like *TSHR*, *PAX8*, and *NKX2-1* [8,40]. Less commonly, DH results from defects in thyroid hormone synthesis, predominantly associated with mutations in the *DUOX2*, *TPO*, and *TG* genes [41–43].

Our study found a high proportion of GIS cases (43%), indicating a possible increased prevalence of CH due to DH. As goiter was not consistently present, in the absence of perchlorate testing and genetic analysis, a definitive diagnosis of DH could not be made.

Populations in which consanguineous marriages are common have reported a rising incidence of CH and DH [44]. Such marriages significantly increase the likelihood of inheriting recessively transmitted genetic mutations, contributing to a higher rate of CH related to mutations in genes like *DUOX2* or *TPO* compared to populations with lower rates of consanguinity [45]. Arab countries with high consanguinity rates also show a relatively high prevalence of CH based on newborn screening programs. For example, CH incidence ranges from 1/1778 in the UAE to 1/2939 in Saudi Arabia [13,28,46]. Furthermore, consanguinity is associated with an increased incidence of congenital abnormalities, including umbilical hernia, congenital heart disease, genitourinary malformations, and cleft palate [8,39,47–49]. However, these malformations are not typically linked to autosomal recessive mutations commonly associated with consanguinity, such as *DUOX2* and *TPO* genes, which are typically linked to isolated thyroid dysfunction. In contrast, autosomal dominant mutations in genes like *PAX8*, *NKX2-1*, and *NKX2-5* are more often associated with syndromic forms or extrathyroidal anomalies [50].

In Algeria, consanguinity is reported in nearly 30% of marriages [51], which may explain the high proportion of gland-in situ cases and of congenital abnormalities.

4.3. Permanent and Transient Forms of CH

The re-evaluation at three years of age revealed that 51% of children with CH tested displayed normal thyroid function, suggesting a substantial number of cases had transient CH. This implies that the global prevalence of CH could include patients with temporary forms of the condition. Previous studies have indeed reported that up to 60% of children on L-T_4 replacement therapy experienced transient hypothyroidism leading to the discontinuation of L-T_4 treatment [52,53]. Approximately 17% to 40% of children diagnosed with CH by NBS programs were later found to have transient hypothyroidism [54]. Several factors may contribute to this finding, including variations in assay threshold, maternal TSH receptor-blocking antibodies, maternal antithyroid drugs, genetic defects such as *DUOX2* mutations, and potential iodine imbalance in the population. However, the underlying mechanism often remains unknown [54,55]. Permanent CH is likely in the presence of TD and of an initial TSH > 100 mU/L [56]. In the absence of these, the need for continuation of L-T_4 replacement therapy should always be reassessed in children being treated for CH at three years of age [8,57].

4.4. Treatment of CH

When CH is diagnosed late, the primary focus is on prompt initiation of treatment and addressing existing complications [58]. The standard treatment for CH involves L-T_4 replacement therapy, typically initiated at a dose of 10–15 mcg/kg/day [57,59,60]. However, findings from our study indicate that 52% of patients received an inadequate initial L-T_4 dose and 15% initiated treatment more than one month after diagnosis. This high proportion of delays and suboptimal initial L-T4 dosing likely reflects a combination of factors, including variability in physician practice, hesitancy to initiate higher doses in very young infants, late referrals, limited access to care, and, in some cases, limited adherence to evolving guidelines at the time of diagnosis. It is clear that the treatment with very low doses may delay thyroid hormone normalization and increase the risk of associated complications [59]. The aim of the treatment is to normalize thyroid function and maintain fT_4 levels in the upper half of the age-specific reference range during the first three years

of life [61]. Even when the diagnosis of CH is delayed, immediate treatment initiation is critical to prevent further deterioration and potentially improve existing symptoms. Some recent studies suggest that an individualized dosing based on the etiology and severity of CH can optimize outcome [62], with children with thyroid dysgenesis generally requiring higher L-T_4 doses than children with DH [4,19].

Regular thyroid function testing (TSH and fT_4) is essential to ensure treatment adequacy and to prevent complications from overtreatment or undertreatment. Overtreatment can lead to adverse effects, including hyperthyroidism, while undertreatment may result in persistent hypothyroidism and increase the likelihood of developmental delay [4,63].

4.5. Neurodevelopmental Data

IQ Findings: The limited number of IQ assessments, 47(16%), was primarily due to the lack of availability of standardized cognitive testing in the majority of participating centers, rather than selective clinical indication. This shows the difficulties in measuring cognitive outcomes in the larger CH population and is a potential selection bias. This highlights the need for more research on how early diagnosis and treatment affect cognitive development. Our study found that children with CH had a mean IQ of 87.1 $\pm$ 15.2, which is lower than the general population norm (100 $\pm$ 15). Additionally, IQs below 70 (11%) were significantly more common among CH patients compared to the general population [9]. In contrast, some follow-up studies suggest that the global IQ in CH children treated early thanks to NBS does not differ from that of controls [64–66].

4.5.1. Educational Outcomes and Cognitive Impairments

In school-aged children, 31% repeated a grade or failed school, indicating notable academic challenges. However, children who received early treatment generally performed within the normal range. Nevertheless, generalized learning difficulties were still found in 20% of CH children [67]. Other studies report mild cognitive impairments, including lower mean IQ scores and subtle deficits in attention, memory, fine motor skills, and quality of life [68–73].

A higher initial dose of levothyroxine combined with very early treatment initiation may lead to better cognitive outcomes [74]. A few patients with severe CH may still have subtle cognitive and motor deficits, and lower educational attainment despite early treatment with a high initial L-T_4 dose [70,74–76]. Moreover, the long-term neurodevelopmental outcomes in patients with CH appear to be associated with the severity of hypothyroidism and the subsequent rapid normalization of TSH [8]. In our study, euthyroidism was achieved in all patients at the time of neurocognitive assessment, and mean scores of both developmental quotient and intelligence quotient were lower than the general population, with differences in primary school performance. Even in CH patients screened at birth, mild non-verbal learning disabilities, and less than satisfactory scores for educational attainment, behavior, and motor skills were reported in children with severe CH [76]. However, the finding of CH patients with subnormal IQ suggests that neurodevelopmental rescue should not be taken for granted even in the era of neonatal screening [64,77]. Children diagnosed and treated after 3 months of age are at high risk of permanent cognitive impairment [18,33]. In our study, a diagnosis after 2 months appears to represent the critical threshold in our ROC analysis, with an OR of 7.3 for developing an intellectual disability. In an Indonesian study, 72% of patients with CH (median age 9 years) had a full-scale IQ score <70 (classified as intellectual disability), with late initiation of treatment specifically correlating with reduced performance IQ [33]. Severe cases (TSH >30 mU/L) are 5–14 times more likely to exhibit developmental delay in cognition and language [78,79]. Despite these challenges,

some follow-up studies demonstrate a positive trajectory in cognitive functioning among many patients with CH, particularly those diagnosed and treated early. In our study, five patients showed improvement in their IQ scores over time. This highlights the importance of early intervention and of consistent follow-up to maximize developmental outcomes [1].

4.5.2. Preventing Delayed Diagnosis and Reducing Cognitive Risks in Congenital Hypothyroidism

Preventing delayed diagnosis and reducing cognitive risks related to CH requires a comprehensive approach involving healthcare systems, provider education, and public awareness [61]. An important advancement in preventive medicine has been the implementation of NBS for CH, which has significantly changed the natural history of this condition [33,80,81]. NBS has been crucial in lowering the incidence of intellectual disability associated with untreated CH [38,82]. Based on available data, it is estimated that approximately 25% of children born with clinically diagnosed CH may have experienced overt disability before the adoption of NBS [12,83]. Early detection through NBS is associated with better neurocognitive outcomes in CH [19,61,83].

4.5.3. Challenges in Timely Screening Implementation

Despite its success, NBS programs face some challenges, including early hospital discharges, which can complicate the timing for blood sample collection. In resource-limited settings, efforts should focus on targeted screening approaches for high-risk infants or the development of more affordable screening methods [10]. Additionally, international organizations can assist in establishing and supporting NBS programs in underserved regions, thereby improving early identification and treatment rates globally [84].

4.5.4. Education and Public Awareness Initiatives

Healthcare Provider Education

Ongoing education for healthcare providers is essential to enhance their ability to recognize, diagnose, and manage CH early in the absence of a national NBS program. This is particularly relevant for primary-care providers, who are often the first to encounter subtle signs of CH in infants who were not screened at birth. Regular training programs should focus on identifying subclinical presentations, interpreting screening results, and understanding updated treatment protocols.

Public Awareness Campaigns

Educational campaigns targeting parents and caregivers are also vital. These efforts should emphasize the importance of NBS and raise awareness about the signs of thyroid dysfunction in infants and children, such as delayed growth, feeding difficulties, or developmental delay. Reaching underserved communities—where healthcare access is often limited—requires tailored communication strategies to ensure equitable access to information.

5. Limitations

The study has several limitations. First, its retrospective design introduces potential biases, including incomplete data and reliance on clinical records. Second, the non-exhaustive recruitment of patients may limit the generalizability of the findings. Third, the small number of IQ evaluations (n = 47) restricts the ability to draw definitive conclusions about neurodevelopmental outcomes. Future prospective studies with larger sample sizes and standardized neurodevelopmental assessments are needed to address these limitations.

6. Conclusions

This study highlights the critical challenges in managing CH in Algeria, including delayed diagnosis, suboptimal treatment practices, and the impact of consanguinity on disease etiology. The findings underscore the urgent need for universal NBS to facilitate early diagnosis and treatment, as well as adherence to international guidelines for L-T_4 dosing. Public health interventions to reduce consanguinity and improve access to genetic counseling should also be prioritized. Preventing delayed diagnosis of CH and minimizing its cognitive impact depend on a robust infrastructure for NBS, adequate provider training, and community education initiatives. While NBS has significantly reduced the burden of intellectual disability associated with CH, challenges remain, particularly in resource-limited regions. Finally, further research is needed to evaluate the impact of these interventions on neurodevelopmental outcomes in Algerian children with CH.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijns11030078/s1, Table S1: Clinical characteristics at presentation; Table S2: Age at diagnosis according to the cause of referral; Table S3: Assessment of the Initial treatment with Levothyroxine; Table S4: Risk factor of Neurodevelopmental delay; Figure S1: Clinical signs and symptoms of congenital hypothyroidism found at diagnosis; Figure S2: Age at diagnosis in months according to the cause of referral; Figure S3: Dose L-T4 treatment at initiation by age.

Author Contributions: A.D. and A.L. had full access to all of the data in the study and take responsibility for the integrity and the accuracy of the data analysis. Conceptualization, A.D. and A.L.; methodology, A.D. and A.L.; investigation, A.D. and A.L.; software, A.D.; validation, A.D., A.L. and G.V.V.; formal analysis, A.D.; investigation, A.D., Y.O., K.B., S.K., F.B., M.B. (Mimouna Bessahraoui), N.S., L.D., K.M., K.B.N., H.A., M.B. (Meriem Bensalah), D.L., F.A., D.D., S.D., M.S.D., N.R., M.O., G.V.V. and A.L.; data curation, A.D.; writing—original draft preparation, A.D. and A.L.; writing—review and editing, A.D., A.L. and G.V.V.; visualization, A.D. and A.L.; supervision, A.D., A.L. and G.V.V.; project administration, A.D. and A.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Van Vliet, G.; Deladoëy, J. Diagnosis, Treatment and Outcome of Congenital Hypothyroidism. *Endocr. Dev.* **2014**, *26*, 50–59. [CrossRef]
2. Grant, D.B.; Smith, I.; Fuggle, P.W.; Tokar, S.; Chapple, J. Congenital Hypothyroidism Detected by Neonatal Screening: Relationship between Biochemical Severity and Early Clinical Features. *Arch. Dis. Child.* **1992**, *67*, 87–90. [CrossRef]
3. Alm, J.; Larsson, A.; Zetterstrom, R. Congenital Hypothyroidism in Sweden: Psychomotor Development in Patients Detected by Clinical Signs and Symptoms. *Acta Pædiatrica* **1981**, *70*, 907–912. [CrossRef]
4. He, S.; Ma, X.; Yang, J.; Li, L. Levothyroxine Treatment for Congenital Hypothyroidism Based on Thyroid Function: A 10-Year Clinical Retrospective Study. *BMC Endocr. Disord.* **2022**, *22*, 142. [CrossRef]
5. Donaldson, M.; Jones, J. Optimising Outcome in Congenital Hypothyroidism; Current Opinions on Best Practice in Initial Assessment and Subsequent Management. *JCRPE J. Clin. Res. Pediatr. Endocrinol.* **2013**, *5*, 13–22. [CrossRef]

6. Fasfous, A.F.; Daugherty, J.C. Cultural Considerations in Neuropsychological Assessment of Arab Populations. In *Cultural Diversity in Neuropsychological Assessment*; Routledge: New York, NY, USA, 2022; pp. 135–150.
7. Fasfous, A.F.; Al-Joudi, H.F.; Puente, A.E.; Pérez-García, M. Neuropsychological Measures in the Arab World: A Systematic Review. *Neuropsychol. Rev.* **2017**, *27*, 158–173. [CrossRef]
8. Van Trotsenburg, P.; Stoupa, A.; Léger, J.; Rohrer, T.; Peters, C.; Fugazzola, L.; Cassio, A.; Heinrichs, C.; Beauloye, V.; Pohlenz, J.; et al. Congenital Hypothyroidism: A 2020–2021 Consensus Guidelines Update—An ENDO-European Reference Network Initiative Endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. *Thyroid* **2021**, *31*, 387–419. [CrossRef]
9. Grosse, S.D.; Van Vliet, G. Prevention of Intellectual Disability through Screening for Congenital Hypothyroidism: How Much and at What Level? *Arch. Dis. Child.* **2011**, *96*, 374–379. [CrossRef]
10. Grob, F.; Lain, S.; Olivieri, A. Newborn Screening for Primary Congenital Hypothyroidism: Past, Present and Future. *Eur. Thyroid J.* **2025**, *14*, e240358. [CrossRef]
11. Kaye, C.I.; Schaefer, G.B.; Bull, M.J.; Enns, G.M.; Gruen, J.R.; Hersh, J.H.; Mendelsohn, N.J.; Saal, H.M. Introduction to the Newborn Screening Fact Sheets. *Pediatrics* **2006**, *118*, 1304–1312. [CrossRef]
12. Fisher, D.A.; Dussault, J.H.; Foley, T.P.; Klein, A.H.; LaFranchi, S.; Larsen, P.R.; Mitchell, M.L.; Murphey, W.H.; Walfish, P.G. Screening for Congenital Hypothyroidism: Results of Screening One Million North American Infants. *J. Pediatr.* **1979**, *94*, 700–705. [CrossRef]
13. Rastogi, M.V.; LaFranchi, S.H. Congenital Hypothyroidism. *Orphanet J. Rare Dis.* **2010**, *5*, 17. [CrossRef] [PubMed]
14. Pezzuti, I.L.; De Lima, P.P.; Dias, V.M.A. Hipotireoidismo Congĉnito: Perfil Clínico Dos Recém-Nascidos Identificados Pelo Programa de Triagem Neonatal de Minas Gerais. *J. Pediatr.* **2009**, *85*, 72–79. [CrossRef]
15. Raiti, S.; Newns, G.H. Cretinism: Early Diagnosis Its Relation to Mental Prognosis. *Arch. Dis. Child.* **1971**, *46*, 692–694. [CrossRef]
16. Alm, J.; Larsson, A.; Zetterström, R. Congenital Hypothyroidism in Sweden Incidence and Age at Diagnosis. *Acta Pædiatrica* **1978**, *67*, 1–3. [CrossRef]
17. LaFranchi, S. Congenital Hypothyroidism: Etiologies, Diagnosis, and Management. *Thyroid* **1999**, *9*, 735–740. [CrossRef]
18. Yunis, K.A.; Nasr, M.R.; Lepejian, G.; Najjar, S.; Daher, R. Short Report: False-Negative Primary Neonatal Thyroid Screening: The Need for Clinical Vigilance and Secondary Screening. *J. Med. Screen.* **2003**, *10*, 2–4. [CrossRef]
19. Nagendra, L.; Bhavani, N.; Pavithran, P.V.; Shenoy, M.; Menon, U.V.; Abraham, N.; Nair, V.; Kumar, H. Etiological Profile, Targeted Levothyroxine Dosing and Impact of Partial Newborn Screening in Congenital Hypothyroidism—A Single Centre Experience. *Indian J. Endocrinol. Metab.* **2023**, *27*, 445–449. [CrossRef] [PubMed]
20. De Jonge, G.A. Congenital Hypothyroidism in the Netherlands. *Lancet* **1976**, *308*, 143. [CrossRef]
21. Wolter, R.; Noël, P.; De Cock, P.; Craen, M.; Ernould, C.; Malvaux, P.; Verstraeten, F.; Simons, J.; Mertens, S.; van Broeck, N.; et al. Neuropsychological Study in Treated Thyroid Dysgenesis. *Acta Paediatr.* **1979**, *68*, 41–46. [CrossRef]
22. Jacobsen, B.B.; Brandt, N.J. Congenital Hypothyroidism in Denmark. *Arch. Dis. Child.* **1981**, *56*, 134–136. [CrossRef]
23. Tarim, O.F.; Yordam, N. Congenital Hypothyroidism in Turkey: A Retrospective Evaluation of 1000 Cases. *Turk. J. Pediatr.* **1992**, *34*, 197–202. [PubMed]
24. Nasheiti NA Childhood Hypothyroidism in Iraq: A Retrospective Study. *Int. J. Endocrinol. Metab.* **2005**, *3*, 136–139.
25. Chen, C.Y.; Lee, K.T.; Lee, C.T.C.; Ter Lai, W.; Huang, Y. Bin Epidemiology and Clinical Characteristics of Congenital Hypothyroidism in an Asian Population: A Nationwide Population-Based Study. *J. Epidemiol.* **2013**, *23*, 85–94. [CrossRef]
26. Niang, B.; Fall, A.L.; Ba, I.D.; Keita, Y.; Ly, I.D.; Ba, A.; Thiongane, A.; Ndongo, A.A.; Boiro, D.; Thiam, L.; et al. Congenital Hypothyroidism in Dakar: About 28 Cases. *Pan Afr. Med. J.* **2016**, *25*, 46. [CrossRef]
27. Deliana, M.; Batubara, J.R.; Tridjaja, B.; Pulungan, A.B. Hipotiroidisme Kongenital Di Bagian Ilmu Kesehatan Anak RS Ciptomangunkusumo Jakarta, Tahun 1992-2002. *Sari Pediatr.* **2016**, *5*, 79. [CrossRef]
28. Saoud, M.; Al-Fahoum, S.; Kabalan, Y. Congenital Hypothyroidism: A Five-Year Retrospective Study at Children's University Hospital, Damascus, Syria. *Qatar Med. J.* **2019**, *2019*, 7. [CrossRef]
29. Kahssay, M.; Ngwiri, T. High Yield of Congenital Hypothyroidism among Infants Attending Children Hospital, Nairobi, Kenya. Facility Based Study in the Absence of Newborn Screening. *J. Pediatr. Endocrinol. Metab.* **2025**, *38*, 51–57. [CrossRef]
30. Klein, A.H.; Meltzer, S.; Kenny, F.M. Improved Prognosis in Congenital Bypothyroidism Treated before Age Three Months. *J. Pediatr.* **1972**, *81*, 912–915. [CrossRef]
31. Gulshan, A.; Tahmina, B.; Fouzia, M.; Mizanur, R. Neurodevelopmental Outcome of Congenital Hypothyroidism in Children between 1-5 Years of Age. *Bangladesh J. Med. Sci.* **2011**, *10*, 245–251. [CrossRef]
32. John, T.; Anirudhan, D. Physical Growth and Intellectual Function of Children with Congenital Hypothyroidism: An Observational Study. *Sri Lanka J. Child Health* **2023**, *52*, 148–154. [CrossRef]

33. Pulungan, A.B.; Oldenkamp, M.E.; Van Trotsenburg, A.S.P.; Windarti, W.; Gunardi, H. Effect of Delayed Diagnosis and Treatment of Congenital Hypothyroidism on Intelligence and Quality of Life: An Observational Study. *Med. J. Indones.* **2019**, *28*, 396–401. [CrossRef]
34. Léger, J.; Larroque, B.; Norton, J. Influence of Severity of Congenital Hypothyroidism and Adequacy of Treatment on School Achievement in Young Adolescents: A Population-Based Cohort Study. *Acta Paediatr. Int. J. Paediatr.* **2001**, *90*, 1249–1256. [CrossRef]
35. Gunnerbeck, A.; Lundholm, C.; von Döbeln, U.; Zetterström, R.H.; Almqvist, C.; Nordenström, A. Congenital Hypothyroidism and School Achievement in Adolescence: A Population-Based Sibling Control Study. *J. Pediatr.* **2024**, *275*, 114240. [CrossRef]
36. Kreisner, E.; Schermann, L.; Camargo-Neto, E.; Gross, J.L. Predictors of Intellectual Outcome in a Cohort of Brazilian Children with Congenital Hypothyroidism. *Clin. Endocrinol.* **2004**, *60*, 250–255. [CrossRef]
37. Selva, K.A.; Harper, A.; Downs, A.; Blasco, P.A.; LaFranchi, S.H. Neurodevelopmental Outcomes in Congenital Hypothyroidism: Comparison of Initial T4 Dose and Time to Reach Target T4 and TSH. *J. Pediatr.* **2005**, *147*, 775–780. [CrossRef]
38. Léger, J.; Ecosse, E.; Roussey, M.; Lanoë, J.L.; Larroque, B. Subtle Health Impairment and Socioeducational Attainment in Young Adult Patients with Congenital Hypothyroidism Diagnosed by Neonatal Screening: A Longitudinal Population-Based Cohort Study. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 1771–1782. [CrossRef]
39. Pramono, L.A.; Yuwono, A. Late Diagnosis of Congenital Hypothyroidism in Young Adult. *Acta Med. Indones.* **2019**, *51*, 272–274.
40. Makretskaya, N.; Bezlepkina, O.; Kolodkina, A.; Kiyaev, A.; Vasilyev, E.V.; Petrov, V.; Kalinenkova, S.; Malievsky, O.; Dedov, I.I.; Tiulpakov, A. High Frequency of Mutations in'dyshormonogenesis Genes' in Severe Congenital Hypothyroidism. *PLoS ONE* **2018**, *13*, e0204323. [CrossRef]
41. Shin, J.H.; Kim, H.Y.; Kim, Y.M.; Lee, H.; Bae, M.H.; Park, K.H.; Lee, S.M.; Kwak, M.J. Genetic Evaluation of Congenital Hypothyroidism with Gland in Situ Using Targeted Exome Sequencing. *Ann. Clin. Lab. Sci.* **2021**, *51*, 73–81.
42. Li, L.; Li, X.; Wang, X.; Han, M.; Zhao, D.; Wang, F.; Liu, S. Mutation Screening of Eight Genes and Comparison of the Clinical Data in a Chinese Cohort with Congenital Hypothyroidism. *Endocrine* **2023**, *79*, 125–134. [CrossRef]
43. Bruellman, R.J.; Watanabe, Y.; Ebrhim, R.S.; Creech, M.K.; Abdullah, M.A.; Dumitrescu, A.M.; Refetoff, S.; Weiss, R.E. Increased Prevalence of TG and TPO Mutations in Sudanese Children with Congenital Hypothyroidism. *J. Clin. Endocrinol. Metab.* **2020**, *105*, 1564–1572. [CrossRef]
44. Albert, B.B.; Cutfield, W.S.; Webster, D.; Carll, J.; Derraik, J.G.B.; Jefferies, C.; Gunn, A.J.; Hofman, P.L. Etiology of Increasing Incidence of Congenital Hypothyroidism in New Zealand from 1993–2010. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 3155–3160. [CrossRef]
45. Hall, S.; Hutchesson, A.; Kirk, J. Congenital Hypothyroidism, Seasonality and Consanguinity in the West Midlands, England. *Acta Paediatr.* **1999**, *88*, 212–215. [CrossRef]
46. Golbahar, J.; Al-Khayyat, H.; Hassan, B.; Agab, W.; Hassan, E.; Darwish, A. Neonatal Screening for Congenital Hypothyroidism: A Retrospective Hospital Based Study from Bahrain. *J. Pediatr. Endocrinol. Metab.* **2010**, *23*, 39–44. [CrossRef]
47. Bundey, S.; Alam, H. A Five-Year Prospective Study of the Health of Children in Different Ethnic Groups, with Particular Reference to the Effect of Inbreeding. *Eur. J. Hum. Genet.* **1993**, *1*, 206–219. [CrossRef] [PubMed]
48. Kumar, J.; Gordillo, R.; Kaskel, F.J.; Druschel, C.M.; Woroniecki, R.P. Increased Prevalence of Renal and Urinary Tract Anomalies in Children with Congenital Hypothyroidism. *J. Pediatr.* **2009**, *154*, 263–266. [CrossRef] [PubMed]
49. Danner, E.; Jääskeläinen, J.; Niuro, L.; Huopio, H.; Niinikoski, H.; Viikari, L.; Kero, J.; Sund, R. Comorbidity in Congenital Hypothyroidism—A Nationwide, Population-Based Cohort Study. *J. Clin. Endocrinol. Metab.* **2023**, *108*, e1695–e1701. [CrossRef]
50. Stoupa, A.; Carré, A.; Polak, M.; Szinnai, G.; Schoenmakers, N. Genetics of Primary Congenital Hypothyroidism: Three Decades of Discoveries and Persisting Etiological Challenges. *Eur. Thyroid J.* **2025**, *14*, e240348. [CrossRef] [PubMed]
51. Benallègue, A.; Kedji, F. Consanguinité et Santé Publique. Une Étude Algérienne. *Arch. Fr. Pediatr.* **1984**, *41*, 435–440.
52. Nagasaki, K.; Sato, H.; Sasaki, S.; Nyuzuki, H.; Shibata, N.; Sawano, K.; Hiroshima, S.; Asami, T. Re-Evaluation of the Prevalence of Permanent Congenital Hypothyroidism in Niigata, Japan: A Retrospective Study. *Int. J. Neonatal Screen.* **2021**, *7*, 27. [CrossRef]
53. Matejek, N.; Tittel, S.R.; Haberland, H.; Rohrer, T.; Busemann, E.M.; Jorch, N.; Schwab, K.O.; Wölfle, J.; Holl, R.W.; Bettendorf, M. Predictors of Transient Congenital Primary Hypothyroidism: Data from the German Registry for Congenital Hypothyroidism (AQUAPE "HypoDok"). *Eur. J. Pediatr.* **2021**, *180*, 2401–2408. [CrossRef] [PubMed]
54. Kanike, N.; Davis, A.; Shekhawat, P.S. Transient Hypothyroidism in the Newborn: To Treat or Not to Treat. *Transl. Pediatr.* **2017**, *6*, 349–350. [CrossRef] [PubMed]
55. Peters, C.; Schoenmakers, N. MECHANISMS IN ENDOCRINOLOGY: The Pathophysiology of Transient Congenital Hypothyroidism. *Eur. J. Endocrinol.* **2022**, *187*, R1–R16. [CrossRef]
56. Schoen, E.J.; Clapp, W.; To, T.T.; Fireman, B.H. The Key Role of Newborn Thyroid Scintigraphy with Isotopic Iodide (123I) in Defining and Managing Congenital Hypothyroidism. *Pediatrics* **2004**, *114*, e683–e688. [CrossRef] [PubMed]

57. Itonaga, T.; Hasegawa, Y.; Higuchi, S.; Satoh, M.; Sawada, H.; Shimura, K.; Takahashi, I.; Takubo, N.; Nagasaki, K. Knowns and Unknowns about Congenital Hypothyroidism: 2022 Update. *Clin. Pediatr. Endocrinol.* **2023**, *32*, 11–25. [CrossRef]
58. Simoneau-Roy, J.; Marti, S.; Deal, C.; Huot, C.; Robaey, P.; Van Vliet, G. Cognition and Behavior at School Entry in Children with Congenital Hypothyroidism Treated Early with High-Dose Levothyroxine. *J. Pediatr.* **2004**, *144*, 747–752. [CrossRef]
59. Rahmani, K.; Yarahmadi, S.; Etemad, K.; Koosha, A.; Mehrabi, Y.; Aghang, N.; Soori, H. Congenital Hypothyroidism: Optimal Initial Dosage and Time of Initiation of Treatment: A Systematic Review. *Int. J. Endocrinol. Metab.* **2016**, *14*, e36080. [CrossRef]
60. Aleksander, P.E.; Brückner-Spieler, M.; Stoehr, A.-M.; Lankes, E.; Kühnen, P.; Schnabel, D.; Ernert, A.; Stäblein, W.; Craig, M.E.; Blankenstein, O.; et al. Mean High-Dose l-Thyroxine Treatment Is Efficient and Safe to Achieve a Normal IQ in Young Adult Patients With Congenital Hypothyroidism. *J. Clin. Endocrinol. Metab.* **2018**, *103*, 1459–1469. [CrossRef]
61. Rose, S.R.; Wassner, A.J.; Wintergerst, K.A.; Yayah-Jones, N.-H.; Hopkin, R.J.; Chuang, J.; Smith, J.R.; Abell, K.; LaFranchi, S.H.; Wintergerst, K.A.; et al. Congenital Hypothyroidism: Screening and Management. *Pediatrics* **2023**, *151*, e2022060419. [CrossRef]
62. Esposito, A.; Vigone, M.C.; Polizzi, M.; Wasniewska, M.G.; Cassio, A.; Mussa, A.; Gastaldi, R.; Di Mase, R.; Vincenzi, G.; Pozzi, C.; et al. Effect of Initial Levothyroxine Dose on Neurodevelopmental and Growth Outcomes in Children with Congenital Hypothyroidism. *Front. Endocrinol.* **2022**, *13*, 923448. [CrossRef]
63. Vigone, M.C.; Ortolano, R.; Vincenzi, G.; Pozzi, C.; Ratti, M.; Assirelli, V.; Vissani, S.; Cavarzere, P.; Mussa, A.; Gastaldi, R.; et al. Treatment of Congenital Hypothyroidism: Comparison between L-Thyroxine Oral Solution and Tablet Formulations up to 3 Years of Age. *Eur. J. Endocrinol.* **2022**, *186*, 45–52. [CrossRef]
64. Vulsma, T.; Gons, M.H.; de Vijlder, J.J.M. Maternal-Fetal Transfer of Thyroxine in Congenital Hypothyroidism Due to a Total Organification Defect or Thyroid Agenesis. *N. Engl. J. Med.* **1989**, *321*, 13–16. [CrossRef] [PubMed]
65. LaFranchi, S. Congenital Hypothyroidism: A Newborn Screening Success Story? *Endocrinologist* **1994**, *4*, 477–486. [CrossRef]
66. Collaborative, N.E.C.H. Neonatal Hypothyroidism Screening: Status of Patients at 6 Years of Age. *J. Pediatr.* **1985**, *107*, 915–919. [CrossRef] [PubMed]
67. Bargagna, S.; Dinetti, D.; Pinchera, A.; Marcheschi, M.; Montanelli, L.; Presciuttini, S.; Chiovato, L. School Attainments in Children with Congenital Hypothyroidism Detected by Neonatal Screening and Treated Early in Life. *Eur. J. Endocrinol.* **1999**, *140*, 407–413. [CrossRef]
68. Hulse, J.A. Outcome for Congenital Hypothyroidism. *Arch. Dis. Child.* **1984**, *59*, 23–29. [CrossRef]
69. Rovet, J.; Ehrlich, R.; Sorbara, D. Intellectual Outcome in Children with Fetal Hypothyroidism. *J. Pediatr.* **1987**, *110*, 700–704. [CrossRef]
70. Murphy, G.H.; Hulse, J.A.; Smith, I.; Grant, D.B. Congenital Hypothyroidism: Physiological and Psychological Factors in Early Development. *J. Child Psychol. Psychiatry* **1990**, *31*, 711–725. [CrossRef]
71. Fuggle, P.W.; Grant, D.B.; Smith, I.; Murphy, G. Intelligence, Motor Skills and Behaviour at 5 Years in Early-Treated Congenital Hypothyroidism. *Eur. J. Pediatr.* **1991**, *150*, 570–574. [CrossRef]
72. Glorieux, J.; Dussault, J.; Van Vliet, G. Intellectual Development at Age 12 Years of Children with Congenital Hypothyroidism Diagnosed by Neonatal Screening. *J. Pediatr.* **1992**, *121*, 581–584. [CrossRef]
73. Bargagna, S.; Chiovato, L.; Dinetti, D.; Montanelli, L.; Giachetti, C.; Romolini, E.; Marcheschi, M.; Pinchera, A. Neuropsychological Development in a Child with Early-Treated Congenital Hypothyroidism as Compared with Her Unaffected Identical Twin. *Eur. J. Endocrinol.* **1997**, *136*, 100–104. [CrossRef]
74. Simons, W.F.; Fuggle, P.W.; Grant, D.B.; Smith, I. Intellectual Development at 10 Years in Early Treated Congenital Hypothyroidism. *Arch. Dis. Child.* **1994**, *71*, 232–234. [CrossRef] [PubMed]
75. Hauri-Hohl, A.; Dusoczky, N.; Dimitropoulos, A.; Leuchter, R.H.-V.; Molinari, L.; Caflisch, J.; Jenni, O.G.; Latal, B. Impaired Neuromotor Outcome in School-Age Children With Congenital Hypothyroidism Receiving Early High-Dose Substitution Treatment. *Pediatr. Res.* **2011**, *70*, 614–618. [CrossRef] [PubMed]
76. Simons, W.F.; Fuggle, P.W.; Grant, D.B.; Smith, I. Educational Progress, Behaviour, and Motor Skills at 10 Years in Early Treated Congenital Hypothyroidism. *Arch. Dis. Child.* **1997**, *77*, 219–222. [CrossRef] [PubMed]
77. Uchida, K.; Suzuki, M. Congenital Hypothyroidism and Brain Development: Association With Other Psychiatric Disorders. *Front. Neurosci.* **2021**, *15*, 772382. [CrossRef]
78. Romero, M.A.; Goto, M.M.F.; D'Ouro, M.P.C.; Lima, M.C.M.P.; Dutra, V.F.; Mendes-dos-Santos, C.T.; Santos, D.C.C. Analysis of Motor, Cognitive and Language Performance of Infants Undergoing Treatment for Congenital Hypothyroidism. *J. Pediatr.* **2025**, *101*, 172–178. [CrossRef]
79. Habib, M.M.F.; Faddan, H.H.I.A.; Metwalley, K.A.; Ismail, T.A.A.M. Growth and Developmental Milestones in Children with Congenital Hypothyroidism Attending Assiut Health Insurance Clinic. *Egypt. J. Community Med.* **2022**, *40*, 233–242. [CrossRef]
80. Rovet, J.F. Congenital Hypothyroidism: Long-Term Outcome. *Thyroid* **1999**, *9*, 741–748. [CrossRef]

81. Léger, J. Congenital Hypothyroidism: A Clinical Update of Long-Term Outcome in Young Adults. *Eur. J. Endocrinol.* **2015**, *172*, R67–R77. [CrossRef]
82. Smith, D.W.; Klein, A.M.; Henderson, J.R.; Myrianthopoulos, N.C. Congenital Hypothyroidism-Signs and Symptoms in the Newborn Period. *J. Pediatr.* **1975**, *87*, 958–962. [CrossRef]
83. Ahmad, N.; Irfan, A.; Al Saedi, S. Congenital Hypothyroidism: Screening, Diagnosis, Management, and Outcome. *J. Clin. Neonatol.* **2017**, *6*, 64. [CrossRef]
84. Hannon, H.; Therrell, B.; World Health Organization. Hereditary Diseases Programme. In *Guidelines on the Prevention and Control of Congenital Hypothyroidism / Prepared by Harry Hannon, Brad Therrell*; World Health Organization: Geneva, Switzerland, 1990.

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Implementation of Neonatal Screening Program for Congenital Hypothyroidism in Eastern Morocco

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Abstract

Congenital hypothyroidism (CH) is one of the major preventable causes of intellectual disability. This study evaluates the incidence of CH through a newborn screening (NBS) program in eastern Morocco. A descriptive cross-sectional design was used and heel prick blood samples were collected on blotting paper to measure Thyroid-Stimulating Hormone (TSH) using an immunofluorimetric assay. 4062 newborns were screened (51.3% male, 48.7% female). TSH levels significantly varied by age: newborns sampled before 24 h had a higher median TSH (3.7 μU/mL [0.10–28.90]) compared to those sampled at 24 h or more (2.1 μU/mL [0.10–32.30]; $p < 0.001$). Using age-specific cut-off values, 18 suspected CH cases were recalled (recall rate: 0.44%). Among the 16 cases who completed confirmatory testing, 4 had transient hyperthyrotropinemia (HTT), characterized by mildly abnormal serum TSH and T4 levels that normalized spontaneously after few months without treatment. Three cases were diagnosed with CH confirmed at birth with markedly elevated serum TSH concentrations and significantly reduced T4 levels. Consequently, the birth prevalence of CH confirmed at birth was 1:1354 live births. The median preanalytical delay was 6 days (IQR: 3–12) and the TSH result turnaround was 8 days (IQR: 5–15), potentially affecting timely intervention. This first report from eastern Morocco confirms the relevance of neonatal screening but highlights delays that must be addressed to enhance early diagnosis and management.

Keywords: congenital hypothyroidism; newborn screening; eastern Morocco

1. Introduction

Congenital hypothyroidism (CH) is a thyroid hormone deficiency presenting at birth, and it is the most common preventable cause of intellectual disability [1]. Thyroid hormones regulate the function of most organ systems and play a crucial role in children's normal growth and neurodevelopment. Indeed, CH can cause cardiological, neurological, gastrointestinal, and metabolic dysfunctions if not diagnosed and treated. CH may be of thyroidal (primary CH) or central origin (secondary and tertiary hypothyroidism). Primary CH can be caused by thyroid dysgenesis or thyroid dyshormonogenesis [2]. Recent studies have shown that, with the increasing incidence of primary CH, the prevalence of thyroid

dysgenesis and thyroid function defects has changed with around 60% thyroid dysgenesis and 40% functional defects [3].

Most newborns with CH have no clinical signs, and late diagnosis leads to the most severe outcomes, particularly irreversible intellectual disability. Therefore, newborn Thyroid-Stimulating Hormone (TSH) screening is one of the best, most cost-effective tools for preventing intellectual disability in the population, enabling prompt treatment with levothyroxine [4]. In developed countries, the introduction of NBS has largely eliminated neurodevelopmental impairment from CH. However, implementing such a program remains challenging in other countries [5].

For the first time, we carried out a regional NBS study based on measuring TSH in heel prick blood on blotting papers to assess the birth prevalence of CH in eastern Morocco.

2. Materials and Methods

This is a descriptive cross-sectional study designed to assess the prevalence of CH in newborns, the TSH turnaround time (TAT) and screening timeframes.

All newborns delivered in eastern Morocco and for whom written parental consent was obtained were included in this study. Newborns with incomplete data were excluded.

This study was conducted in eight public maternity wards and three neonatal units of eastern Morocco from March 2024 to December 2024. Newborn heel prick blood samples were collected on labeled blotting papers (PerkinElmer 226, Waltham, MA, USA), and blood samples were dried and sent to the collection point of the Medical Analysis Laboratory at Al Farabi Regional Hospital (ARH) of Oujda for analysis to minimize measurement bias. Furthermore, all suspected CH cases were recalled by telephone at ARH for a venous TSH and free T4 (FT4) quantification as a confirmatory test and underwent a clinical examination by qualified health workers (midwives or physicians) to collect anthropometric data (weight), clinical data (CH symptoms and also other pathologies observed in newborns), and demographic data (age at screening and sex) using a questionnaire.

Quantitative determination of the TSH in the dried blood was performed using an immunofluorimetric method DELFIA Neonatal hTSH kit (PerkinElmer, Waltham, MA, USA) according to manufacturer's instructions. Age-related blood TSH cutoffs were applied to report suspected CH. It is necessary to specify that a cutoff of 20 μU/L blood was used for newborns whose ages were less than or equal to 24 h. Conversely, a cutoff of 15 μU/mL was used for those aged more than 24 h old. These thresholds are based on the national recommendations established by the Moroccan Ministry of Health for neonatal CH screening protocols.

Two distinct situations have been defined based on the results of the confirmatory test. Newborns with mildly elevated or borderline serum TSH and/or T4 levels during the initial confirmatory test, which normalized spontaneously without any medical intervention, were classified as having transient HTT. In contrast, newborns with significantly elevated TSH levels and clearly low T4 concentrations, consistent with primary hypothyroidism, were considered as having CH confirmed at birth.

To assess the timeliness of the CH screening program, we collected dates of birth, sampling, analysis, and result transmission. Dates were collected from medical records to avoid performance bias. The screening program was divided into two phases: Phase 1, from heel prick blood sampling to TSH analysis, and Phase 2, from analysis to the transmission of results. The turnaround time (TAT) was defined as the interval between the sample collection and the transmission of TSH results. For each newborn we calculated TAT, Phase 1, and Phase 2 timeframes.

Statistical Analysis

All data were imported into SPSS software version 25 (IBM Corp., Armonk, NY, USA) for analysis. Descriptive statistics were used to summarize the characteristics of the newborn population, including general data (age, sex, sampling sites, and screening locations), anthropometric and clinical parameters (gestational age, weight, length, symptoms, and associated conditions), and TSH levels. The Kolmogorov–Smirnov test was applied to assess the normality of distribution for continuous variables. As most quantitative data, including TSH values, were not normally distributed, they were expressed as medians with interquartile ranges [Q1–Q3 or IQR], and comparisons were conducted using non-parametric tests. The Mann–Whitney U test was used to compare continuous variables between two independent groups (e.g., TSH levels in newborns sampled before vs. after 24 h). The Kruskal–Wallis test was used for comparing continuous variables across more than two groups (e.g., TSH levels by gestational age category). The Chi-square test Pearson or Fisher's exact test was used to compare categorical variables, depending on expected frequency distributions. The results were considered as statistically significant when $p < 0.05$.

3. Results

3.1. Characteristics of the Newborn Population

During the study period, 4062 newborns were enrolled for CH screening, with an almost equal distribution of males and females (sex ratio male to female of 1.05). Over half (57.9%) of the newborns were less than 24 h old, with a median age [Q1–Q3] at screening of 21 [12–36] hours. In our newborn population, 94.5% were born at full term, 4.4% were born prematurely, and only 0.2% were born after term. The birth weight was 3400 [3000–3800] g without any difference between males and females (Table 1).

Most newborns (88.7%) were managed in the maternity center (including both maternity hospitals and maternity home), and 11.3% were in the neonatal unit. Around two thirds (69%) of deliveries took place in urban areas, whereas 31% occurred in rural areas. The screening for CH was carried out in all provinces of eastern Morocco (Table 1).

Table 1. General data of newborns enrolled for CH screening.

	Total (*N* = 4062)	**Male (*N* = 2085)**	**Female (*N* = 1977)**	***p*-Value**
Age at screening (hours) (*N* = 3982)				
Median [Q1–Q3]	21 [12–36]	22 [12–37]	21 [12–34]	0.011 [a]
<24, *n* (%)	2305 (57.9%)	1139 (55.7%)	1166 (60.2%)	0.004 [b]
≥24, *n* (%)	1677 (42.1%)	907 (44.3%)	770 (39.8%)	
Term pregnancy (weeks) (*N* = 4023)				
<37, *n* (%)	178 (4.4%)	83 (4.0%)	95 (4.8%)	NS [b]
37 to 41, *n* (%)	3836 (94.5%)	1975 (95.7%)	1861 (94.9%)	
≥41, *n* (%)	9 (0.2%)	5 (0.2%)	4 (0.2%)	
Weight (grams) (*N* = 3834)				
Median [Q1–Q3]	3400 [3000–3800]	3400 [3050–3850]	3400 [3000–3800]	NS [a]
≥2500, *n* (%)	3590 (93.6%)	1855 (94.5%)	1735 (93.8%)	NS [b]
<2500, *n* (%)	244 (6.4%)	129 (6.5%)	37 (6.2%)	
Sampling sites				
Maternity center, *n* (%)	3603 (88.7%)	1815 (87.1%)	1788 (90.4%)	>0.001 [b]
Neonatal unit, *n* (%)	459 (11.3%)	270 (12.9%)	189 (9.6%)	

Table 1. *Cont.*

	Total (*N* = 4062)	Male (*N* = 2085)	Female (*N* = 1977)	*p*-Value
Environment				
Urban, *n* (%)	2784 (69.0%)	1441 (69.3%)	1343 (68.5%)	NS [b]
Rural, *n* (%)	1255 (31.0%)	637 (30.7%)	618 (31.5%)	
Screening locations				
Oujda, *n* (%)	1755 (43.3%)	922 (44.3%)	833 (42.2%)	NS [b]
Nador, *n* (%)	574 (14.2%)	288 (13.8%)	286 (14.5%)	
Jerada, *n* (%)	438 (10.8%)	227 (10.9%)	211 (10.7%)	
Berkane, *n* (%)	309 (7.62%)	152 (7.30%)	157 (7.96%)	
Driouch, *n* (%)	307 (7.57%)	146 (7.01%)	157 (7.96%)	
Guercif, *n* (%)	268 (6.61%)	147 (7.06%)	121 (6.14%)	
Taourirt, *n* (%)	209 (5.15%)	105 (5.04%)	104 (5.27%)	
Figuig, *n* (%)	195 (4.81%)	96 (4.61%)	99 (5.02%)	

[a] Mann–Whitney U test, [b] Fisher exact/Pearson test, NS: not significant.

3.2. TSH Variation Among Newborns

The median TSH value for the 4062 newborns was 3.0 [1.8–4.7] µU/mL. A significant difference was observed between the sexes ($p = 0.01$): females had a median TSH of 2.9 µU/mL [1.7–4.5 µU/mL], while males had a slightly higher median of 3.1 µU/mL [1.9–5.0 µU/mL]. The median TSH level was similar in newborns of low birth weight (<2500 g) (3.1 µU/mL [1.9–4.6]) compared with those weighing ≥ 2500 g (3.0 µU/mL [1.8–4.7]). Regarding the term of pregnancy, the median TSH value was higher in post-term newborns (3.9 µU/mL [2.6–5.3]), followed by full-term newborns (3.0 µU/mL [1.8–4.8]) and premature newborns (2.65 µU/mL [1.5–4.1]). Statistical comparisons were performed for all relevant neonatal variables. No significant associations were found between TSH levels and gestational age (term vs. preterm) or birth weight (<2500 g vs. ≥2500 g), and therefore these results were not emphasized in the main analysis. Interestingly, newborns aged less than 24 h had a median TSH of 3.7 µU/mL [2.3–5.7], whereas those aged 24 h or more showed a lower median of 2.1 µU/mL [1.3–3.4]; this difference was statistically significant ($p < 0.001$) (Table 2).

Table 2. TSH variation among newborns.

	TSH (µU/mL)		*p*-Value
	Median	[Q1–Q3]	
Total (*N* = 4062)	3.0	[1.8–4.7]	
Gender			
Male	3.1	[1.9–5.0]	0.01 [a]
Female	2.9	[1.7–4.5]	
Age (hours)			
<24 h	3.7	[2.3–5.7]	<0.001 [a]
≥24 h	2.1	[1.3–3.4]	
Weight (grams)			
≥2500 g	3.0	[1.8–4.7]	NS [a]
<2500 g	3.1	[1.9–4.6]	
Term of pregnancy (weeks)			
<37	2.6	[1.5–4.1]	NS [b]
37 to 41	3.0	[1.8–4.8]	
≥41	3.9	[2.6–5.3]	

[a] Mann–Whitney U test, [b] KRUSKAL Wallis test, TSH: Thyroid-Stimulating Hormone, NS: not significant.

3.3. CH Screening in Eastern Morocco

By applying the provided age-related cut-offs, 18 newborns screened positive and were suspected of having CH (recall rate of 0.44%)—12 male and 6 female. Among them, 13 cases were less than 24 h old and 5 cases were aged 24 h or more. Of the 18 cases recalled at ARH for confirmatory testing, 16 presented for analysis. Among them, 4 newborns had mildly elevated or borderline TSH and/or T4 values at the initial confirmatory test, which normalized during follow-up without the need for treatment. These cases were classified as transient hyperthyrotropinemia (HTT), reflecting a temporary and self-limiting disturbance in thyroid function (median TSH 8.89 μU/mL [6.26–9.13 μU/mL]; median T4 20.73 pmol/L [19.27–22.31 pmol/L]), which normalized upon subsequent testing during follow-up without requiring hormone replacement therapy. These cases were classified as transient HTT. In contrast, confirmatory tests identified 3 cases with a diagnosis of CH confirmed at birth, who had a very high TSH and low T4 levels (median TSH = 180 μU/mL [176–196 μU/mL] and median T4 = 3.8 pmol/l [3.78–4.08 pmol/l]). These results showed that the birth prevalence of CH confirmed at birth was 0.07% (3/4062), or 1 case per 1354 live births. For the remaining 9 newborns, confirmatory test results were entirely within normal limits from the outset, indicating false-positive screening results, representing 0.22% (Table 3).

Table 3. Outcomes of the CH screening program in eastern Morocco.

	Total (*N* = 4062)		**Male (*N* = 2085)**		**Female (*N* = 1977)**		***p*-Value [a]**
	n	**(%)**	***n***	**(%)**	***n***	**(%)**	
Recall rate	18	(0.44%)	12	(0.58%)	6	(0.30%)	NS
Newborns < 24 h	13	(0.32%)	9	(0.43%)	4	(0.20%)	NS
Newborns ≥24 h	5	(0.12%)	3	(0.14%)	2	(0.10%)	NS
Negative confirmatory test	9	(0.22%)	6	(0.29%)	3	(0.15%)	NS
Transient HTT	4	(0.10%)	2	(0.10%)	2	(0.10%)	NS
CH confirmed at birth	3	(0.07%)	2	(0.10%)	1	(0.05%)	NS

[a] Fisher exact/Pearson test, NS: not significant, CH: congenital hypothyroidism, HTT: hyperthyrotropinemia.

3.4. Profile of CH Newborns Suspected of Having CH

The median age at screening was significantly higher in confirmed CH cases (100 h [40–120]) compared to transient HTT cases (9 h [6–11]) and newborns who had a negative confirmatory test (12 h [7–20]). Overall, 62.5% of recalled newborns were screened before 24 h of life. All transient HTT cases were screened before 24 h of life and initially showed mild hormonal abnormalities that later normalized. In contrast, only one out of three newborns with confirmed CH at birth was screened before 24 h; the remaining two were screened later and had clearly abnormal hormone levels requiring treatment.

Regarding birth weight, the median weight was similar in all groups: 3100 g [2500–3400] for newborns with a diagnosis of CH confirmed at birth, 3150 g [2900–3600] for those with transient HTT, and 3120 g [2700–3800] for those with negative confirmatory test. Low birth weight (<2500 g) was reported in 25% of newborns recalled for confirmatory testing, with comparable proportions between those with a diagnosis of CH confirmed at birth and newborns who had a negative confirmatory test, while no transient HTT case had low birth weight.

A thorough examination showed that some clinical signs were observed exclusively in newborns with a diagnosis of CH confirmed at birth; hypotonia and feeding difficulties were present in 100% of cases and mottled skin in 66.7%. Other signs, including

cyanosis, constipation, puffy face, and macroglossia, were each only reported in individual cases. These symptoms were not observed in transient HTT cases or negative groups, except for cyanosis and mottled skin, which were present in few cases with negative confirmatory tests (11.1% and 22.2% respectively).

In addition, 6 out of 16 newborns recalled for confirmatory testing presented with associated pathologies including malformation, cardiopathy, and nephropathy. We also observed that comorbidities were not exclusive to cases with a diagnosis of CH confirmed at birth. In fact, 33.3% of newborns with a negative confirmation test had associated pathologies, compared with 66.6% of those with a confirmed CH diagnosis at birth (Table 4).

Table 4. Clinical profile of newborns recalled for confirmatory testing after positive CH screening.

	Screen-Positive Newborns (*N* = 16)	**Negative Confirmatory Test (*N* = 9)**	**Transient Hyperthyrotropinemia (*N* = 4)**	**Confirmed CH at Birth (*N* = 3)**
Age at screening (hours)				
Median [Q1–Q3]	13 [8–24]	12 [7–20]	9 [6–11]	100 [40–120]
<24, *n* (%)	10 (62.5%)	5 (55.6%)	4 (100%)	1 (33.3%)
≥24, *n* (%)	6 (37.5%)	4 (44.4%)	0 (0.0%)	2 (66.7%)
Weight (grams)				
Median [Q1–Q3]	3100 [2800–3600]	3120 [2700–3800]	3150 [2900–3600]	3100 [2500–3400]
≥2500 *n* (%)	12 (75%)	6 (66.7%)	4 (100%)	2 (33.3%)
<2500, *n* (%)	4 (25%)	3 (33.3%)	0 (0%)	1 (66.7%)
Clinical signs				
Cyanosis	2 (12.5%)	1 (11.1%)	0 (0%)	1 (33.3%)
Mottled skin	4 (25%)	2 (22.2%)	0 (0%)	2 (66.7%)
Hypotonia	3 (18.8%)	0 (0%)	0 (0%)	3 (100%)
Constipation	1 (6.3%)	0 (0%)	0 (0%)	1 (33.3%)
Feeding difficulties	3 (18.8%)	0 (0%)	0 (0%)	3 (100%)
Puffy face	1 (6.3%)	0 (0%)	0 (0%)	1 (33.3%)
Macroglossia	1 (6.3%)	0 (0%)	0 (0%)	1 (33.3%)
Comorbidities				
Malformations	2 (12.5%)	1 (11.1%)	1(25%)	0 (0%)
Cardiopathies	2 (12.5%)	1 (11.1%)	0 (0%)	1 (33.3%)
Nephropathies	2 (12.5%)	1 (11.1%)	0 (0%)	1 (33.3%)

NS: not significant, CH: congenital hypothyroidism.

3.5. Assessment of the Timeliness of the CH Screening Program

For the assessment of the timeliness of the screening program, 1119 random samples were analyzed. The overall median turnaround time was 8 days (IQR: 5–15). By dividing the screening program into two phases, we observed that the timeframe of Phase 1 (from heel prick sampling to analysis) was longer, with a median of 6 days (IQR: 3–12) compared to the timeframe of Phase 2 (from analysis to result transmission) between analysis and transmission (1 day). Interestingly, we noted a distinct variation in the turnaround time and timeframes between Oujda, where the Laboratory of ARH is located, and the other screening locations. Indeed, the lowest TAT median was observed in Oujda (5 days, IQR: 4–8), and the highest TAT medians were observed in Nador (20 days, IQR: 16–28) and Driouch (12 days, IQR: 10–14). Similarly, the timeframe of Phase 1 was shorter in Oujda compared to the other locations. However, the timeframes of Phase 2 were closer from one province to another, with a median of 4 days (IQR: 1–4) observed in Jerada and a median of 1 day in Oujda. Finally, we observed that all screening results, available only one day after the analysis, were transmitted within 1 to 40 days after birth, with a median of 9 days (IQR: 6–17) (Table 5).

Table 5. Assessment of the timeliness of the CH screening program.

	Timeframes (Days)							
	Birth to Transmission		TAT		Sampling to Analysis		Analysis to Transmission	
	Median	(IQR)	Median	(IQR)	Median	(IQR)	Median	(IQR)
Total (*N* = 1119)	9	(6–17)	8	(5–15)	6	(3–12)	1	(1–4)
Screening locations								
Oujda (*n* = 427)	6	(4–9)	5	(4–8)	4	(2–6)	1	(1–1)
Nador (*n* = 191)	21	(18–29)	20	(16–28)	18	(14–24)	2	(1–4)
Berkane (*n* = 138)	15	(9–18)	13	(8–16)	10	(6–14)	2	(1–4)
Jerada (*n* = 125)	10	(7–15)	9	(6–11)	6	(3–9)	4	(1–4)
Guercif (*n* = 71)	9	(6–14)	8	(5–11)	6	(4–8)	1	(1–2)
Taourirt (*n* = 69)	7	(4–10)	6	(3–8)	4	(2–6)	1	(1–1)
Figuig (*n* = 57)	8	(5–13)	7	(4–11)	5	(3–7)	1	(1–2)
Driouch (*n* = 41)	13	(10–15)	12	(10–14)	9	(6–11)	4	(2–4)

CH: congenital hypothyroidism, TAT: turnaround time, IQR: interquartile range.

4. Discussion

Congenital hypothyroidism is a thyroid hormone deficiency presenting at birth, and it is the most common preventable cause of intellectual disability. Screening all newborns has been recognized as the most effective method to prevent severe morbidities associated with CH [5,6]. Since 2006, the Moroccan Ministry of Health has drawn up an action plan and protocols for newborn CH screening, and in 2012 it launched a regional program in Rabat-Salé. Gradually, this program extended to other regions of the country [7]. In eastern Morocco, the NS program was officially launched in December 2022. For organizational reasons, sampling did not begin until March 2024. This study is the first experience of regional NBS for CH in eastern Morocco aiming to estimate the birth prevalence of CH and assess the timeliness of this program.

Three strategies are used to detect newborns with CH: a primary T4 test strategy, a primary TSH test strategy (based on measuring either TSH in heel prick blood spot or cord blood TSH), and a combined strategy. Currently, the TSH strategy is the most frequently used in screening programs worldwide [1,5]. The present screening protocol involves measuring heel prick blood TSH. By contrast, a combined strategy has been used in other CH screening studies in Morocco [8,9]. According to the guidelines of the American Academy of Pediatrics, to avoid false positive results, CH screening is recommended between 24 and 72 h when TSH levels stabilize after a normal surge that occurs within hours after birth. However, samples collected before 48 h are preferable to avoid any delay in screening in case of early discharge. In addition, samples collected before 24 h necessitate the use of age-specific TSH reference ranges [5]. Given that early discharges are frequent, we screened all newborns just before discharge, respecting a screening age of 5 days or less, and applied age-related cutoffs for TSH level in accordance with the national standards: 20 μU/L for newborns whose ages are less than or equal to 24 h and 15 μU/mL for those aged more than 24 h old.

The prevalence of preterm and low birthweight varies across geographic regions. The global prevalence of preterm is 11.1%, ranging from 5% in European countries to 18% in African countries [10]. The global prevalence of low-birthweight newborns estimated in 2015 is 14.6%, with an average of 17.3% in Asia and 7.2% in developed countries [11,12]. In this screening program, 4.4% of newborns enrolled were premature and 6.4% were low birthweight, aligning with the literature. In line with national recommendations, repeat

TSH screening is advised for preterm and low-birth-weight newborns to detect possible delayed TSH rise. However, in our study, no second screening was conducted for at-risk newborns with normal initial results, which limits our ability to assess CH incidence in this subgroup. In almost all CH screening programs, including the screening program in the Fez region, a female predominance was reported [9,13]. However, no female predominance was found in our study.

Although standard guidelines recommend repeat TSH testing at 15 and 30 days in preterm newborns, twins, and those born to mothers with thyroid disorders, this protocol was not implemented in our cohort. Only newborns with initial TSH above threshold values were recalled for confirmation. Recall rates in different screening programs vary from 0.01 to 13.3% and are generally lower in primary TSH screening programs than in the T4 strategy [14]. The recall rate in our screening program was 0.44%, which is closer to those reported in the Rabat region (0.6%) and the Fez region (0.5%) [8,9]. This higher rate could be explained by lower cutoffs applied here (20–15 mU/μL) and iodine deficiency or excess; the rate can rise from 0.1% in the non-iodine-deficient population to 20% in the iodine-deficient population [15]. Of note, Morocco is considered a moderate iodine-deficiency area [8,9]. Newborns exposed to excess iodine, such as iodine-containing antiseptics, may also develop hypothyroidism [16,17]. Future improvements to the screening program should include systematic follow-up for high-risk newborns to better detect delayed-onset congenital hypothyroidism.

CH is usually sporadic and occurs in 1 in 2000 to 4000 births. In recent decades, an increase in the incidence of CH has been reported in many studies worldwide [18,19]. This increase is thought to be related to a switch in screening strategies, lower TSH cutoffs, changes in ethnic demographics, and an increased number of preterm and low-birthweight newborns screened [3]. Historically, switching from the primary T4 strategy to the primary TSH strategy has led to an increase in the incidence of primary CH [1]. On the other hand, lowering the TSH cutoffs has led to a change in the etiologies of CH, with more mild and transient cases being detected [19,20]. Significant variation in incidence has been observed among ethnicities, with a higher rate in Hispanic (1:1600), Asian (1:1757 to 1:2380), and non-Hispanic white newborns (1:3333) and a lower rate in Black newborns (1:11,000) [21]. A higher incidence has also been reported in multiple pregnancies (10.1: 10,000) [22] and in same-sex twins (1:593) [23,24].

In Morocco, we have no data on the overall birth prevalence of CH, and few studies have been conducted on the topic. The first neonatal screening campaign for CH was carried out in 1996 in the Rabat region, revealing an incidence of 1:1138 [8]. Another study carried out in the Fez region reported an incidence of 1:1952 [9]. In this study, we report a birth prevalence of 1:1354 live births of CH confirmed at birth, which aligns with Moroccan studies. In addition, according to international guidelines on CH, the four newborns with elevated TSH at screening and mildly abnormal or borderline serum TSH and/or T4 values at the initial confirmatory test, which normalized spontaneously without treatment during follow-up, were classified as having transient HTT. These cases are distinct from both confirmed CH and false-positive results, as they reflect a temporary functional disturbance rather than a false alarm or permanent hypothyroidism. Also, the three newborns with high serum TSH and low serum T4 at confirmation cannot be considered cases with permanent CH but rather cases with a diagnosis of CH confirmed at birth, unless a diagnosis of thyroid dysgenesis is provided in the first days of life by means of thyroid scintigraphy and/or ultrasound. In the absence of an ascertained diagnosis of thyroid dysgenesis (agenesis, ectopy, hypoplasia), a re-evaluation of the diagnosis should be performed at 2–3 years of life after withdrawal of the replacement therapy to verify whether

CH confirmed at birth is permanent (re-start therapy) or transient (no therapy). It should be noted that, in a related study exploring maternal and neonatal factors affecting TSH levels in the same region, significantly higher rates of elevated TSH were observed in the province of Figuig (OR = 3.878; $p = 0.024$), possibly linked to high consanguinity rates and other contextual variables [25]. Although this current study did not identify statistically significant geographic variations in confirmed CH cases due to the limited number of diagnoses, these findings support the need for further investigation into province-specific risk factors.

Most newborns with CH have few or no clinical manifestations at birth. Furthermore, symptoms of hypothyroidism are non-specific. As a result, up to 60% of newborns are unaware of their conditions [26]. Studies conducted over the last two decades have also shown that many newborns with CH have concomitant congenital abnormalities such as cardiac and musculoskeletal malformations [27]. CH is also very common in Down's syndrome newborns (1 to 12%) [5]. In Morocco, Oulmaati et al. reported that most newborns with CH had hypotonia (46%), hypothermia (32%), jaundice (34%), lethargy (22%), feeding difficulties (32%), myxedema (15%), macroglossia (15%), dry skin (12%), and wide fontanelle (10%). Moreover, 78% of these newborns had associated pathologies, especially Down's syndrome, Turner's syndrome, cardiopathies, and digestive atresia [28]. In our study, the most common symptoms were hypotonia (42.9%) and feeding difficulties (42.9%), followed by mottled skin (28.6%). The other symptoms represented only 14.3%. In addition, three out of seven cases presented with associated pathologies, including malformation, cardiopathy, and nephropathy. These findings align with previous studies and highlight that NBS is the most effective approach to identifying CH cases.

Neonatal screening programs require the perfect coordination of a multidisciplinary team, robust organization, and consistent funding. In our context, the Medical Analysis Laboratory of ARH is the only laboratory in eastern Morocco to perform TSH quantitation on blotting papers, and for technical and logistical reasons, dried blood samples were sent to the collection point of the laboratory, where TSH quantification was carried out once a week. In addition, all results were available the day after analysis. Therefore, timeframes between sampling and analysis, as well as those between analysis and transmission, are crucial for therapy. Our time monitoring showed that the timeframes of screening phases were longer, especially the pre-analytic delay, resulting in an elevated turnaround time. The variability in TAT observed across provinces highlights significant logistical and structural challenges encountered during the initial phase of the screening program. These include the absence of a dedicated transport system for blood samples, particularly from rural areas; the irregular supply of screening materials; and a shortage of trained healthcare staff at collection sites. Furthermore, the regional reference laboratory operated with very limited human resources, which contributed to processing delays. These constraints, especially prevalent in provinces like Nador and Berkane, may lead to delayed confirmatory testing and late initiation of treatment in newborns with elevated TSH levels, which increases the risk of preventable intellectual disability. This finding is alarming and highlights the need to optimize these processes. Indeed, the routing of blotting papers and results transmission requires strong coordination between midwives, service majors, regional intermediaries, and the laboratory. Strengthening the logistics chain, improving supply continuity, and reinforcing the regional laboratory's capacity are essential to ensure timely and equitable neonatal screening in eastern Morocco. We also suggest that abnormal results should be communicated directly to a designated physician coordinator in each maternity ward or neonatal unit and transferred into the hospital information system.

We are aware that all screened newborns were enrolled in the main public hospitals in each province. Therefore, this program should be implemented in all medical care centers of eastern Morocco to achieve an effective CH screening program. The screening strategy used here could also be a limitation. Each screening strategy has advantages and disadvantages, leading to a different recall rate and CH birth prevalence. Indeed, in the primary TSH approach, central hypothyroidism, hypothyroxinemia, TBG deficiency, and delayed TSH elevation would be missed. By contrast, the T4 approach is more sensitive in detecting rare cases of hypothalamic–pituitary hypothyroidism. However, the TSH approach has a higher specificity with a smaller recall rate than the T4 program. However, while the combined TSH and T4 strategy is the ideal approach, it is not cost-effective [29]. Reducing the cutoff to 5 mU/L in the UK enabled the identification of cases that would not be detected with the recommended cutoffs [30]. Further studies are thus needed to define optimal cutoffs in our newborn population. Along with this, the presence of only one laboratory in the Oriental region, an area of more than 90,000 km^2, explains why the preanalytical delays are very large. This can delay the handling of confirmed CH cases at the right time. As such, it is preferable to equip each province with a laboratory even if the expenses of screening would be higher, as the consequences of intellectual disability are far more costly.

Furthermore, human errors are inevitable and can lead to invalid results and an increased recall rate. Ongoing training of midwives and technicians should be considered to reduce non-compliant samples and technical errors. However, given the complexity and uniqueness of each context and the specific needs of each user, the transfer of knowledge must be innovative and relevant in the pedagogical strategies used [31].

5. Conclusions

To the best of our knowledge, this study provides the first comprehensive insight into CH screening in eastern Morocco, reporting a birth prevalence of 1:1354 live births for CH confirmed at birth. However, distinguishing between transient and permanent forms of CH remains doubtful due to the absence of follow-up tests that accurately describe long-term results.

Although the program supports the relevance of neonatal CH screening in eastern Morocco, our findings highlight significant operational challenges that compromise its overall effectiveness. Further research, including longitudinal cohort studies, is essential to optimize screening protocols. In addition, improving infrastructure and streamlining logistics will be essential to ensure equitable implementation of the neonatal screening program across all provinces.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to their ongoing use in a complementary analysis currently in progress.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

CH	Congenital hypothyroidism
TSH	Thyroid-Stimulating Hormone
ARH	Al Farabi Regional Hospital
TAT	Turnaround time

References

1. Ford, G.; LaFranchi, S.H. Screening for congenital hypothyroidism: A worldwide view of strategies. *Best Pract. Res. Clin. Endocrinol. Metab.* **2014**, *28*, 175–187. [CrossRef]
2. Wassner, A.J. Pediatric hypothyroidism: Diagnosis and treatment. *Paediatr. Drugs* **2017**, *19*, 291–301. [CrossRef]
3. Cherella, C.E.; Wassner, A.J. Congenital hypothyroidism: Insights into pathogenesis and treatment. *Int. J. Pediatr. Endocrinol.* **2017**, *2017*, 11. [CrossRef]
4. Vulsma, T.; Gons, M.H.; de Vijlder, J.J. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid agenesis. *N. Engl. J. Med.* **1989**, *321*, 13–16. [CrossRef]
5. Rose, S.R.; Wassner, A.J.; Wintergerst, K.A.; Yayah-Jones, N.H.; Hopkin, R.J.; Chuang, J.; Smith, J.R.; Abell, K.; LaFranchi, S.H.; Bethin, K.E.; et al. Congenital hypothyroidism: Screening and management. *Pediatrics* **2023**, *151*, e2022060420. [CrossRef]
6. Goyal, C.; Naqvi, W. Developmental delay in congenital hypothyroidism. *Pan Afr. Med. J.* **2021**, *38*, 165. [CrossRef]
7. Skrinska, V.; Khneisser, I.; Schielen, P.; Loeber, G. Introducing and expanding newborn screening in the MENA region. *Int. J. Neonatal Screen.* **2020**, *6*, 12. [CrossRef] [PubMed]
8. Zahidi, A.; Thimou, A.; Ibnmajah, M.; EI Abbadi, N.; Mestassi, M.; Draoui, M.; Lamdouar-Bouazzaoui, N. Depistage Neonatal de L'Hypothyroidie Congenitale Par Dosage de la TSH et de la T4. *Maroc. Med.* **2002**, *24*, 4–7. [CrossRef]
9. Maniar, S.; Amor, C.; Bijjou, A. Screening of congenital hyperthyroidism in Morocco: A pilot study. *East Mediterr. Health J.* **2019**, *24*, 1066–1073. [CrossRef]
10. Blencowe, H.; Cousens, S.; Oestergaard, M.Z.; Chou, D.; Moller, A.B.; Narwal, R.; Adler, A.; Garcia, C.V.; Rohde, S.; Say, L.; et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: A systematic analysis and implications. *Lancet* **2012**, *379*, 2162–2172. [CrossRef]
11. Krasevec, J.; Blencowe, H.; Coffey, C.; Okwaraji, Y.B.; Estevez, D.; Stevens, G.A.; Ohuma, E.O.; Conkle, J.; Gatica-Domínguez, G.; Bradley, E.; et al. Study protocol for UNICEF and WHO estimates of global, regional, and national low birthweight prevalence for 2000 to 2020. *Gates Open Res.* **2022**, *6*, 80. [CrossRef] [PubMed]
12. United Nations Children's Fund (UNICEF); World Health Organization (WHO). *UNICEF-WHO Low Birthweight Estimates: Levels and Trends 2000–2015*; World Health Organization: Geneva, Switzerland, 2019.
13. Bowden, S.A.; Goldis, M. Congenital Hypothyroidism. In *StatPearls [Internet]*; StatPearls Publishing: Treasure Island, FL, USA, 2023.
14. Mehran, L.; Khalili, D.; Yarahmadi, S.; Amouzegar, A.; Mojarrad, M.; Ajang, N.; Azizi, F. Worldwide recall rate in newborn screening programs for congenital hypothyroidism. *Int. J. Endocrinol. Metab.* **2017**, *15*, e55451. [CrossRef] [PubMed]
15. Ordoukhani, A.; Mirsaiid Ghazi, A.A.; Hajipour, R.; Mirmiran, P.; Hedayati, M.; Azizi, F. Screening for congenital hypothyroidism: Before and after iodine supplementation in Iran. *Iran. J. Endocrinol. Metab.* **2000**, *2*, 93–98.
16. Chanoine, J.P.; Boulvain, M.; Bourdoux, P.; Pardou, A.; Van Thi, H.V.; Ermans, A.M.; Delange, F. Increased recall rate at screening for congenital hypothyroidism in breast fed infants born to iodine overloaded mothers. *Arch. Dis. Child.* **1988**, *63*, 1207–1210. [CrossRef]
17. Koga, Y.; Sano, H.; Kikukawa, Y.; Ishigouoka, T.; Kawamura, M. Effect on neonatal thyroid function of povidone-iodine used on mothers during perinatal period. *J. Obstet. Gynaecol.* **1995**, *21*, 581–585. [CrossRef]
18. Harris, K.B.; Pass, K.A. Increase in congenital hypothyroidism in New York State and in the United States. *Mol. Genet. Metab.* **2007**, *91*, 268–277. [CrossRef]

19. Corbetta, C.; Weber, G.; Cortinovis, F.; Calebiro, D.; Passoni, A.; Vigone, M.C.; Beck-Peccoz, P.; Chiumello, G.; Persani, L. A 7-year experience with low blood TSH cutoff levels for neonatal screening reveals an unsuspected frequency of congenital hypothyroidism (CH). *Clin. Endocrinol.* **2009**, *71*, 739–745. [CrossRef]
20. Albert, B.B.; Cutfield, W.S.; Webster, D.; Carll, J.; Derraik, J.G.; Jefferies, C.; Gunn, A.J.; Hofman, P.L. Etiology of increasing incidence of congenital hypothyroidism in New Zealand from 1993–2010. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 3155–3160. [CrossRef]
21. Hinton, C.F.; Harris, K.B.; Borgfeld, L.; Drummond-Borg, M.; Eaton, R.; Lorey, F.; Therrell, B.L.; Wallace, J.; Pass, K.A. Trends in incidence rates of congenital hypothyroidism related to select demographic factors: Data from the United States, California, Massachusetts, New York, and Texas. *Pediatrics* **2010**, *125* (Suppl. S2), S37–S47. [CrossRef]
22. Olivieri, A.; Medda, E.; De Angelis, S.; Valensise, H.; De Felice, M.; Fazzini, C.; Cascino, I.; Cordeddu, V.; Sorcini, M.; Stazi, M.A. High risk of congenital hypothyroidism in multiple pregnancies. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 3141–3147. [CrossRef]
23. Jiang, X.; Huang, Y.L.; Feng, Y.; Tang, F.; Jia, X.F.; Chen, Q.Y.; Tang, C.F.; Liu, S.C.; Li, B.; Zheng, R.D.; et al. Same-sex twins have a high incidence of congenital hypothyroidism and a high probability to be missed at newborn screening. *Clin. Chim. Acta* **2020**, *502*, 111–115. [CrossRef]
24. Perry, R.; Heinrichs, C.; Bourdoux, P.; Khoury, K.; Szöts, F.; Dussault, J.H.; Vassart, G.; Van Vliet, G. Discordance of monozygotic twins for thyroid dysgenesis: Implications for screening and for molecular pathophysiology. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 4072–4077. [CrossRef]
25. Wahoud, F.; Essadki, S.; Elmsellem, H.; Amrani, R. Effect of maternal factors on the results of neonatal screening for congenital hypothyroidism in the eastern region of Morocco. *E3S Web Conf.* **2025**, *632*, 02014. [CrossRef]
26. Alzahrani, A.S.; Al Mourad, M.; Hafez, K.; Almaghamsy, A.M.; Alamri, F.A.; Al Juhani, N.R.; Alhazmi, A.S.; Saeedi, M.Y.; Alsefri, S.; Alzahrani, M.D.; et al. Diagnosis and Management of Hypothyroidism in Gulf Cooperation Council (GCC) Countries. *Adv. Ther.* **2020**, *37*, 3097–3111. [CrossRef] [PubMed]
27. Razavi, Z.; Yavarikia, A.; Torabian, S. Congenital anomalies in infant with congenital hypothyroidism. *Oman Med. J.* **2012**, *27*, 364–367. [CrossRef] [PubMed]
28. Oulmaati, A.; Hmami, F.; Hida, M.; Bouharrou, A. [High prevalence of hypothyroidism among children hospitalized in a neonatal intensive care unit in Morocco]. *Arch. Pediatr.* **2016**, *23*, 105–106. [CrossRef] [PubMed]
29. Büyükgebiz, A. Newborn Screening for Congenital Hypothyroidism. *J. Clin. Res. Pediatr. Endocrinol.* **2013**, *5*, 8–12. [CrossRef]
30. Pryce, R.A.; Gregory, J.W.; Warner, J.T.; John, R.; Bradley, D.; Evans, C. Is the current threshold level for screening for congenital hypothyroidism too high? An audit of the clinical evaluation, confirmatory diagnostic tests and treatment of infants with increased blood spot thyroid-stimulating hormone concentrations identified on newborn blood spot screening in Wales. *Arch. Dis. Child.* **2007**, *92*, 1048. [CrossRef]
31. Lemieux-Doutreloux, S. La Simulation In Situ, Une Stratégie pour Favoriser un Meilleur Transfert des Apprentissages dans la Pratique aux Soins Intensifs [Internet]. 2023. Available online: https://savoirs.usherbrooke.ca/bitstream/handle/11143/21464/lemieux-doutreloux_Sara_MSc_2023.pdf?sequence=4 (accessed on 5 August 2024).

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Incidence of Congenital Hypothyroidism Is Increasing in Chile

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Abstract

Congenital hypothyroidism (CH) is a leading preventable cause of neurocognitive impairment. Its incidence appears to be rising in several countries. We analysed 27 years of newborn-screening data (1997–2023) from the largest Chilean screening centre, covering 3,225,216 newborns (51.1% of national births), to characterise temporal trends and potential drivers of CH incidence. Annual CH incidence was modelled with Prais–Winsten regression to correct for first-order autocorrelation; additional models assessed trends in gestational age, sex, biochemical markers, and aetiological subtypes. We identified 1550 CH cases, giving a mean incidence of 4.9 per 10,000 live births and a significant yearly increase of 0.067 per 10,000 (95 % CI 0.037–0.098; $p < 0.001$). Mild cases (confirmation TSH < 20 mU/L) rose (+0.89 percentage points per year; $p = 0.002$). The program's recall was low (0.05%). Over time, screening and diagnostic TSH values declined, total and free T4 concentrations rose, gestational age at diagnosis fell, and a shift from thyroid ectopy toward hypoplasia emerged; no regional differences were detected. The sustained increase in CH incidence, alongside falling TSH thresholds and growing detection of in situ glands, suggests enhanced recognition of milder disease. Ongoing surveillance should integrate environmental, iodine-nutrition, and genetic factors to clarify the causes of this trend.

Keywords: congenital hypothyroidism; newborn screening; incidence trends; time-series analysis; thyroid-stimulating hormone; mild CH; Chile

1. Introduction

Newborn screening (NBS) for congenital hypothyroidism (CH) is a critical public health intervention aimed at preventing intellectual disability associated with untreated CH. Early diagnosis and prompt initiation of levothyroxine therapy can lead to normal neurocognitive development into adulthood [1].

The global prevalence of CH has increased since 1969 [2–6], a trend that may reflect the widespread adoption of NBS programs and the lowering of TSH diagnostic thresholds [4,7,8]. Additionally, the profile of newborns screened for primary CH in developed nations has undergone significant changes with an increase in preterm and multiple births, and infants admitted to NICU [9,10]. Further contributing factors may include iodine deficiency [11] and ethnic differences [12].

In Chile, the NBS program for CH began as a pilot initiative in 1984, led by the National Institute of Nutrition and Food Technology (INTA) and the Ministry of Health. In 1992, the

National Program for Mass Screening of Phenylketonuria and CH was officially launched and gradually expanded to achieve nationwide coverage by 1998 [13]. Currently, the NBS program covers 99% of newborns. This high coverage has proven effective in ensuring normal neurocognitive outcomes in children diagnosed with CH [14]. To date, however, there are no published data on the incidence or etiological distribution of CH in the Chilean population. The objective of this study was to analyse temporal trends in the incidence of CH in Chile between 1997 and 2023.

2. Materials and Methods

The Chilean NBS program has always been based on the measurement of neonatal TSH. Two NBS centres in Chile receive the special blood collection paper cards from the babies born in the public health system (Centres 1 and 2), representing around of 80% of the country's births, and process 76% and 24% of the samples coming from 16 regions of the nation, respectively [13]. Whole-blood TSH on dried blood samples is collected on special blood collection paper. According to national guidelines, the timing of sample collection is based on gestational age: in term infants ($\geq$37 weeks), the sample is obtained between 40 and 48 hours of life; in late preterm infants (36–36$^{6}/_{7}$ weeks), the sample is collected on the 7th day of life; and in preterm infants ($\leq$35 weeks), two samples are collected—one at 7 days and another at 15 days of life. These time intervals have remained unchanged throughout the entire duration of the screening program. Information related to CH diagnoses is collected, including sex, age at sampling, screening and confirmatory TSH levels, and thyroid scintigraphy results when available. Data on gestational age has been systematically recorded since 2009.

Between 1998 and 2006, TSH screening was performed using IRMA DPC (Immunoradiometric Assay, CLINITEST, Diagnostic Products Corporation, Los Angeles, CA, USA), and in 2006 switched to the Delfia® time-resolved fluorometry system (PerkinElmer) (Waltham, MA, USA). In May 2018, the laboratory adopted the fully automated GSP platform (PerkinElmer) (Waltham, MA, USA). To harmonise results after transitioning from AutoDelfia to GSP platforms, a re-evaluation of TSH cutoff values was performed using ROC curve analysis on a dataset of 55,717 newborns screened between May and October 2018. This analysis, identified a new TSH action cutoff of $\geq$13 µIU/mL (serum-equivalent), compared to the previous threshold of 15 µIU/mL.

Under the Delfia method (Figure 1), an initial TSH measurement < 13 µIU/mL is deemed screen-negative; values $\geq$ 13 µIU/mL trigger two repeat assays on separate card punches, with the mean of all three readings dictating next steps: <14 µIU/mL is negative, 14.0–23.9 µIU/mL prompts a second-card retest, and $\geq$24.0 µIU/mL leads directly to confirmatory serum TSH/free T4. If the second card mean is $\geq$15 µIU/mL, the newborn is referred; otherwise, no action is taken.

With the GSP platform (Figure 2), samples with initial TSH $\geq$ 10 µIU/mL undergo duplicate reanalysis: if either repeat is $\geq$13 µIU/mL, referral for confirmatory testing occurs; if both are <13 µIU/mL, the screen is negative.

Throughout the study period, TSH concentrations were reported as serum-equivalent values, using a standardised correction factor based on an assumed haematocrit of 55% (1 µU/mL in whole blood = 2.22 µU/mL in serum).

A diagnosis of CH is confirmed when confirmatory sample serum TSH is >10 mU/L and total T_4 is <10 µg/dL. In cases where free T_4 (FT_4) levels are within the reference range of the specific assay employed by each centre, a diagnosis of hyperthyrotropinemia (HT) is made. When available, thyroid scintigraphy using 99mTc is performed to determine the underlying aetiology.

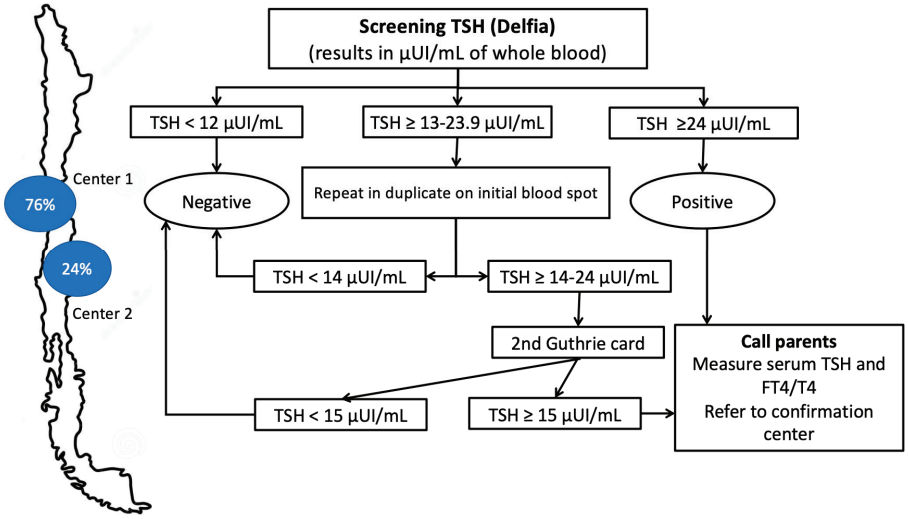

Figure 1. Screening algorithm for CH in Chile (Delfia® time-resolved fluorometry system, 2006–2018).

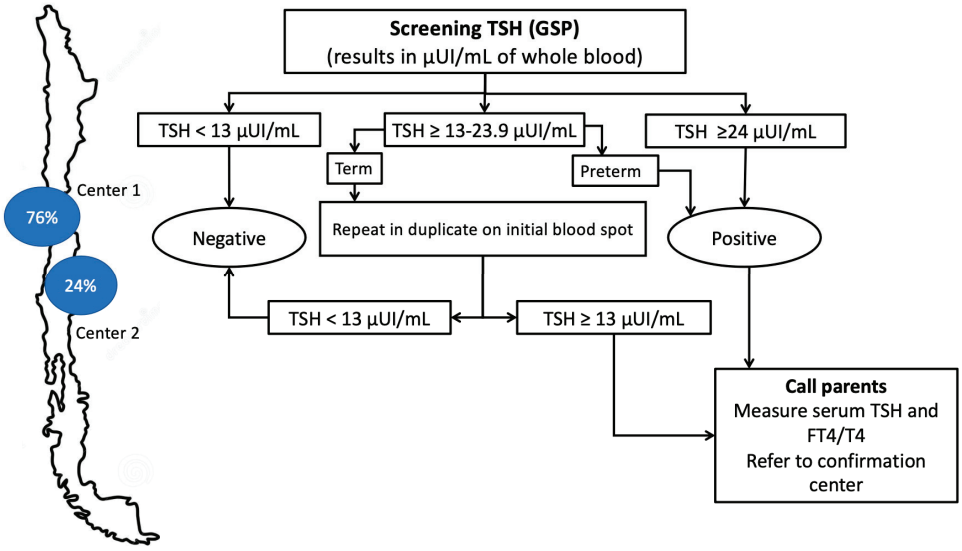

Figure 2. Screening algorithm for CH in Chile (GSP platform, 2018–2023).

A retrospective review was conducted using data from the national NBS program, including infants born within the Chilean public health system between 1997 and 2023, whose special blood collection paper cards were processed at Centre 1. This center receives samples from 11 of Chile's 16 regions, representing approximately 51.1% of all newborns in the country.

The recall rate was defined as the proportion of newborns recalled for a repeat sample or confirmatory test—owing to elevated TSH, inadequate sample, or analytical interference—relative to all newborns with a valid initial specimen. It was calculated as: Recall rate (%) = (Recalled newborns/Total newborns screened) × 100, with exact 95% confidence intervals derived by the Clopper–Pearson method.

Statistical Analysis

Time-trend analyses were performed to describe changes in CH incidence and their potential determinants from 1997 to 2021. Data from 1992 to 1996 were excluded because nationwide screening was still in its pilot phase and lacked full population coverage. First-order autocorrelation in the yearly series was handled with Prais–Winsten regression. The primary outcome was the annual incidence of confirmed CH cases.

Time trends in the incidence of CH were evaluated using Prais–Winsten regression models, with calendar year as the independent variable. In addition to analyzing overall incidence, additional models assessed changes over time in gestational age, sex distribution, age at diagnosis, and hormone concentrations, including screening (whole-blood) and confirmatory (serum) TSH, as well as total and free T4 levels. To investigate whether the observed increase in incidence was driven by mild cases, we conducted two separate analyses: (i) modeling the annual incidence of mild CH, defined by a screening TSH concentration < 20 μUI/mL, and (ii) modeling the yearly proportion of mild cases among all confirmed diagnoses. Conversely, to examine trends in more severe CH, we calculated the annual number of cases with screening TSH $\geq$ 20 μUI/mL and applied a linear regression model to determine whether the frequency of these non-mild cases changed over time. Finally, additional Prais–Winsten models were applied to assess trends in the distribution of aetiologic subtypes (e.g., ectopy, athyreosis, hypoplasia).

For each administrative region, annual incidence was calculated as Incidence = Confirmed CH cases $\times$ 10,000. Regional heterogeneity was assessed with a mixed-effects linear model that treated region as a fixed effect and year as a random intercept, thereby accounting for clustering over time.

Sex-specific differences in CH aetiology were examined among cases with definitive scintigraphic classifications (ectopy, athyreosis, hypoplasia, goitre, normal scan) using Fisher's exact test; cases with missing sex were excluded. All analyses were conducted on yearly aggregated data in Stata 17 (StataCorp, College Station, TX, USA), with statistical significance set at $p < 0.05$.

3. Results

3.1. Overall Incidence (1997–2023)

Between 1997 and 2023, 3,225,216 newborns (51.1% of Chilean live births) were screened for congenital hypothyroidism (CH) at Centre 1. A total of 1550 cases were confirmed, yielding a mean incidence of 4.9 per 10,000 live births, rising from 3.91 in 1997 to 5.75 in 2023 (Figure 3).

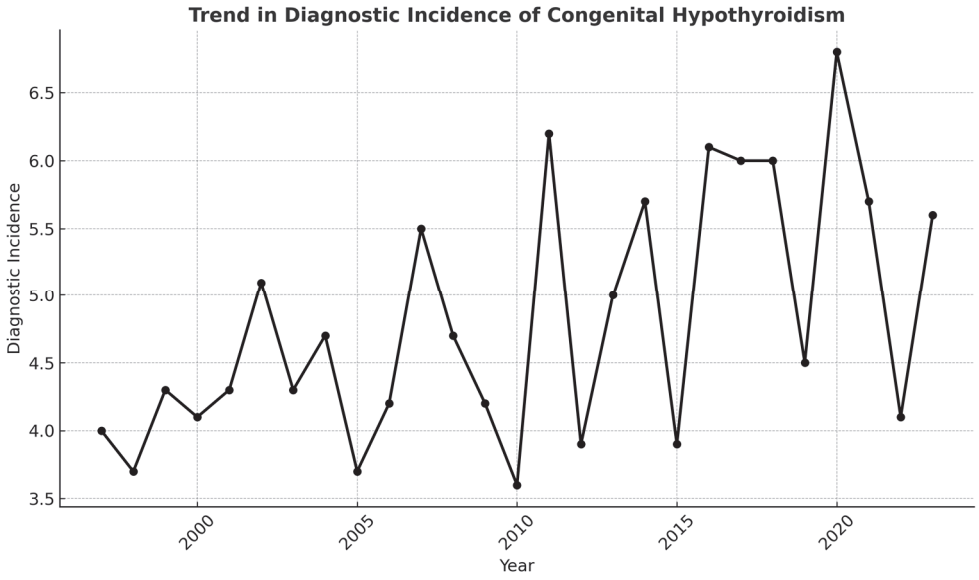

Figure 3. Overall annual incidence rate of CH per 10,000 live births (Chile, 1997–2023).

3.2. Diagnostic Characteristics

Sixty per cent of affected infants were female. The median TSH value at screening was 117.6 μUI/mL (IQR: 65.7–172.0), and at confirmatory serum testing it was 99.2 μUI/mL (IQR: 59.4–100.0). The median total T4 concentration was 5.43 μg/dL (IQR: 4.25–8.00), and the median free T4 concentration was 0.90 ng/dL (IQR: 0.78–1.07). Scintigraphy was not obtained in 58.8 % of cases, which were classified as "unknown" aetiology. Among the remainder, ectopy was most frequent, followed by athyreosis, goitre and hypoplasia (14.8%, 8.4%, 9% and 6%, respectively; Table 1).

Table 1. Number of CH cases by aetiology and overall incidence rates per year, Chile, 1997–2023.

Year	Screened Newborns (*n*)	CH Cases (*n*)	Incidence Rate (per 10,000 Live Births) (Mean)	Ectopy (*n*,%)	Athyreosis (*n*,%)	Hypoplasia (*n*,%)	Goitre (*n*,%)	Normal (*n*,%)	Unknown (*n*,%)
1997	125,245	49	3.91	14 (28)	3 (6)	1 (3)	12 (25)	0 (0)	19 (39)
1998	145,086	53	3.65	15 (28)	13 (25)	4 (8)	3 (6)	0 (0)	17 (33)
1999	141,139	62	4.39	20 (33)	7 (12)	3 (5)	4 (7)	1 (2)	25 (40)
2000	141,093	57	4.04	15 (27)	5 (9)	1 (2)	13 (22)	2 (4)	21 (36)
2001	138,369	61	4.41	12 (20)	6 (10)	4 (6)	9 (14)	1 (2)	29 (48)
2002	137,282	69	5.03	14 (21)	5 (7)	5 (7)	11 (16)	0 (0)	34 (49)
2003	130,707	57	4.36	9 (15)	10 (17)	1 (2)	2 (4)	2 (4)	32 (57)
2004	123,565	58	4.69	5 (9)	5 (9)	0 (0)	3 (5)	0 (0)	45 (77)
2005	120,896	45	3.72	1 (3)	1 (3)	0 (0)	0 (0)	0 (0)	42 (94)
2006	118,802	50	4.21	6 (11)	2 (3)	2 (3)	2 (5)	2 (3)	38 (76)
2007	122,408	68	5.56	4 (6)	0 (0)	1 (2)	1 (2)	0 (0)	61 (90)
2008	123,392	58	4.7	11 (19)	11 (19)	3 (6)	5 (9)	2 (4)	26 (44)
2009	125,383	52	4.15	6 (12)	1 (2)	3 (5)	5 (10)	0 (0)	37 (71)
2010	126,444	44	3.48	10 (22)	2 (5)	3 (7)	4 (9)	3 (7)	22 (49)
2011	121,420	75	6.18	10 (14)	9 (12)	5 (7)	7 (9)	2 (3)	41 (55)
2012	118,081	52	4.4	3 (5)	4 (8)	3 (6)	6 (12)	2 (4)	34 (65)
2013	116,496	58	4.98	7 (12)	7 (12)	1 (2)	3 (6)	1 (2)	39 (67)
2014	119,336	68	5.7	7 (11)	8 (12)	4 (6)	4 (6)	0 (0)	44 (65)
2015	115,322	74	6.42	12 (16)	6 (8)	4 (5)	7 (10)	0 (0)	45 (61)
2016	108,394	43	3.97	5 (12)	1 (2)	1 (2)	3 (7)	0 (0)	33 (76)
2017	108,911	66	6.06	5 (7)	5 (7)	11 (16)	4 (6)	1 (2)	40 (60)
2018	120,561	73	6.06	9 (12)	3 (4)	8 (11)	9 (12)	3 (4)	41 (56)
2019	113,095	53	4.69	5 (9)	4 (7)	7 (14)	5 (10)	5 (10)	26 (49)
2020	96,040	66	6.87	7 (10)	3 (5)	9 (13)	5 (7)	5 (8)	37 (56)
2021	83,934	49	5.84	4 (9)	3 (6)	6 (13)	3 (7)	3 (7)	28 (58)
2022	93,355	38	4.07						
2023	90,460	52	5.75						
Total	3,225,216	1550	4.9	216 (14.8)	124 (8.5)	90 (6.2)	130 (8.9)	35 (4.5)	856 (58.6)

3.3. Diagnostic Performance

The recall rate was 0.05% (95% CI 4.7–5.2 per 10,000). Of the 1604 screen-positive newborns, 10 lacked complete follow-up and were excluded from performance metrics. The overall positive predictive value (PPV) was 32% (95% CI 30–35%). Stratified by severity, the PPV for severe CH was 58% (95% CI 53–63%), while the PPV for mild CH was 14% (95% CI 12–16%). For comparison, the recall rate during the RIA period was 0.047% (95% CI: 3.6–6.2 per 10,000), indicating similar program performance over time.

3.4. Time-Trend Analyses

Prais–Winsten autoregressive regression demonstrated a significant annual increase of 0.067 cases per 10,000 live births (95% CI 0.037–0.098; $p < 0.001$; $R^2 = 0.55$). Mild cases (confirmation TSH < 20 mUI/L) rose by 0.069 per 10,000 per year (95% CI 0.028–0.109; $p = 0.002$; $R^2 = 0.29$) (Figure 4), and their share of all CH diagnoses increased by 0.89 percentage points annually (95% CI 0.37–1.40; $p = 0.002$; $R^2 = 0.31$). Severe cases (screening TSH $\geq$ 20 μUI/mL) decreased significantly over time ($\beta = -0.015$; $p = 0.017$), suggesting a decline in the incidence of clinically overt CH.

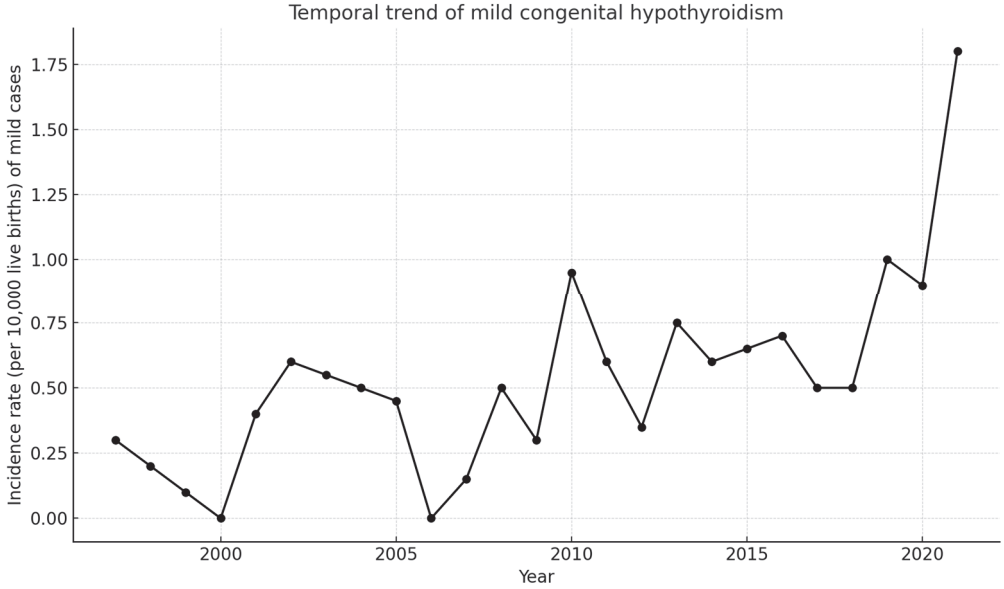

Figure 4. Annual incidence rate of mild cases of CH per 10,000 live births (Chile, 1997–2023).

3.5. Regional Variation

Incidence by region of the country (cases per 10,000 = [confirmed CH/newborns screened] × 10,000) showed no significant differences in a mixed-effects model with region as a fixed effect and year as a random effect (all $p > 0.05$).

3.6. Aetiological Trends and Sex Distribution

The proportion of ectopy and athyreosis decreased ($\beta = -0.007$, $p = 0.019$ and -0.0027, 0.092, respectively) whereas hypoplasia increased ($\beta = 0.0037$; all $p \leq 0.050$). Ectopy showed strong female predominance (72% female, $p < 0.001$), while hypoplasia, goitre and normal scans were more common in males (61%, 59%, and 72% male; $p = 0.005$, 0.019, <0.001, respectively). Dysgenesis (ectopy + athyreosis) had a female/male ratio of 2.47, versus 0.79 for other aetiologies ($p < 0.001$).

3.7. Biochemical Trends

Screening TSH and diagnostic serum TSH both declined over time ($\beta = -8.54$ μUI/mL and -3.93 mU/L per year; $p < 0.001$), whereas total T4 and free T4 rose ($\beta = 0.25$ and 0.03 μg/dL per year; $p \leq 0.002$).

3.8. Multivariable Analysis and Prematurity

In a multivariable Prais–Winsten model including gestational age, sex, aetiology and hormone concentrations, none independently explained the secular rise in incidence.

Prematurity (data available 2009–2023) increased non-significantly over time (β = 0.0098; $p = 0.13$) and was not associated with CH incidence (β = 3.97; $p = 0.31$).

4. Discussion

This study demonstrates a significant upward trend in the diagnostic incidence of CH in Chile over the past two decades. A consistent increase of approximately 0.07 cases per 10,000 live births annually ($p < 0.001$) was observed. This finding aligns with reports from other countries that have documented rising CH incidence over time. In Ireland, the incidence of CH has significantly increased over the past 37 years. with the rate rising from 0.27 cases per 1000 live births between 1979 and 1991 to 0.65 cases per 1000 live births between 2005 and 2016 [15]. Similarly, in China, the incidence increased from 4.01 per 10,000 births in 2012 to 5.77 per 10,000 births in 2019. with factors such as changes in TSH cutoff values and increasing preterm birth rates contributing to this trend [16]. Northern Ireland has also seen a near tripling of CH incidence over 40 years, from 26 cases per 100,000 live births in 1981 to 71 cases per 100,000 in 2019 [17]. In France, the incidence of permanent CH in females increased by 8.9 per year from 2014 to 2019 [18]. A global meta-analysis reported a 52 increase in the prevalence of CH from 1969 to 2020, suggesting that factors such as neonatal screening and changes in diagnostic criteria may be contributing to the rise [2]. However, Finland presents a contrasting picture, where the incidence of CH has remained stable over a 24-year period, indicating that regional differences exist [19].

Several hypotheses have been proposed to explain the increasing incidence of CH worldwide, including improvements in screening sensitivity, changes in diagnostic thresholds [4], and demographic changes [20]. Notably, the increase has been largely attributed to the detection of milder cases, particularly those with in situ thyroid glands. which may not have been diagnosed earlier under more stringent screening criteria [21]. In our analysis, we observed declining trends in both screening and serum TSH concentrations, along with increases in total and free T4 levels. These trends may reflect enhanced detection of milder forms of CH. Additionally, ectopy declined significantly (β = −0.007; $p = 0.012$), whereas the reduction in athyreosis was not significant (β = −0.0027; $p = 0.084$). This trend supports the hypothesis that the increasing incidence of CH may be partly driven by the detection of milder forms of the disease, as previously reported. For instance, in Québec, lowering the TSH cutoff in screening tests led to an increase in the detection of cases with normal-size glands in situ and unknown aetiology, while the incidence of dysgenesis and goitre remained stable [6]. Additionally, the implementation of the GSP platform in 2018 may have increased the detection of milder or borderline cases, contributing to the upward trend in incidence.

Furthermore, the observed decrease in gestational age at approximately one day earlier per year—suggests that an increasing number of cases might being detected among preterm infants. The potential role of prematurity was explored. While the proportion of preterm births appeared to rise slightly, this trend was not statistically significant, and prematurity did not explain the increase in CH incidence in our population. Several studies have highlighted the elevated risk of CH in preterm infants due to their increased survival rates [8] and their unique and dynamic pattern of thyroid hormone levels, explained by the immaturity of the hypothalamic–pituitary–thyroid (HPT) axis; the withdrawal of maternal thyroxine (T4) after birth, exposure to iodine especially in iodine deficient areas; medications; birth weight, and the persistence of fetal metabolism [22]. In China. the increasing rate of preterm births has been identified as a contributing factor to the rising incidence of CH, although the contribution is considered limited compared to other factors like changes in screening practices [16]. In our cohort, 11.7% of confirmed CH cases

occurred in infants born at ≤35 weeks and 6.4% in those born at 36–36$^{6}/_{7}$ weeks. Given that preterm births account for approximately 8–10% of all live births in Chile, these figures suggest a disproportionately higher incidence of CH among preterm infants. However, because our database lacks precise denominators for the total number of preterm births screened each year, we cannot calculate exact incidence rates for this subgroup. This limitation underscores the need for dedicated data collection on gestational age to more accurately assess the contribution of prematurity to CH incidence.

Our findings confirm a clear sex-based difference in the etiological distribution of thyroid dysgenesis, with a pronounced female predominance. This observation is consistent with prior studies suggesting sex-linked susceptibility or inherent developmental differences influencing thyroid morphogenesis and migration [23]. Although the overall proportion of female patients showed a slight but statistically significant decline over time, this trend alone is unlikely to account for the observed increase in CH incidence. Notably, a recent report from France described a rising incidence of permanent CH among females [18], supporting the notion that temporal and regional factors may influence sex-specific trends. Concurrently, we observed a significant increase in cases attributed to thyroid hypoplasia, alongside a decrease in those classified as ectopy or athyreosis. In contrast, dyshormonogenetic presentations (hypoplasia, goitre, and normal scintigraphy) disproportionately affected males, indicating that sex-specific factors—potentially hormonal, placental, or genetic—together with changes in population admixture, may be driving the upward trend in milder CH forms among boys. Unfortunately, because the national screening program collects data only at diagnosis and does not include systematic follow-up, we cannot distinguish whether these milder cases are transient or permanent.

Despite evaluating multiple clinical and biochemical variables, none of the factors examined fully accounted for the upward trend in incidence, suggesting that additional, unmeasured contributors may be at play. These could include changes in iodine status, maternal health conditions, or unidentified environmental factors [24], which underscores the need for future research integrating environmental, nutritional, and genetic data.

5. Conclusions

In summary, we observed a sustained increase in the incidence of CH in Chile, which was associated with a decline in both screening and diagnostic TSH concentration, as well as an increase in cases with in situ thyroid glands. This pattern may reflect a rise in incidence driven by the detection of milder forms of CH. Overall, these findings underscore the importance of continued monitoring of CH incidence and its determinants. Future research should investigate environmental exposures, regional variation in iodine sufficiency, and potential genetic contributions.

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Data Availability Statement: The de-identified individual-level dataset generated during this study is available from the corresponding author on reasonable request. Aggregated incidence data and the Stata code used for analysis are also available on request.

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Abbreviations

The following abbreviations are used in this manuscript:

CH	Congenital Hypothyroidism
NBS	Newborn screening

References

1. Grob, F.; Lain, S.; Olivieri, A. Newborn Screening for Primary Congenital Hypothyroidism: Past, Present and Future. *Eur. Thyroid. J.* **2025**, *14*, e240358. [CrossRef]
2. Liu, L.; He, W.; Zhu, J.; Deng, K.; Tan, H.; Xiang, L.; Yuan, X.; Li, Q.; Huang, M.; Guo, Y.; et al. Global Prevalence of Congenital Hypothyroidism among Neonates from 1969 to 2020: A Systematic Review and Meta-Analysis. *Eur. J. Pediatr.* **2023**, *182*, 2957–2965. [CrossRef]
3. Harris, K.B.; Pass, K.A. Increase in Congenital Hypothyroidism in New York State and in the United States. *Mol. Genet. Metab.* **2007**, *91*, 268–277. [CrossRef]
4. Chiesa, A.; Prieto, L.; Mendez, V.; Papendieck, P.; Calcagno, M.D.L.; Gruñeiro-Papendieck, L. Prevalence and Etiology of Congenital Hypothyroidism Detected through an Argentine Neonatal Screening Program (1997–2010). *Horm. Res. Paediatr.* **2013**, *80*, 185–192. [CrossRef]
5. Mitrovic, K.; Vukovic, R.; Milenkovic, T.; Todorovic, S.; Radivojcevic, J.; Zdravkovic, D. Changes in the Incidence and Etiology of Congenital Hypothyroidism Detected during 30 Years of a Screening Program in Central Serbia. *Eur. J. Pediatr.* **2016**, *175*, 253–259. [CrossRef]
6. Deladoëy, J.; Ruel, J.; Giguère, Y.; Van Vliet, G. Is the Incidence of Congenital Hypothyroidism Really Increasing? A 20-Year Retrospective Population-Based Study in Québec. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 2422–2429. [CrossRef]
7. Mengreli, C.; Kanaka-Gantenbein, C.; Girginoudis, P.; Magiakou, M.A.; Christakopoulou, I.; Giannoulia-Karantana, A.; Chrousos, G.P.; Dacou-Voutetakis, C. Screening for Congenital Hypothyroidism: The Significance of Threshold Limit in False-Negative Results. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 4283–4290. [CrossRef]
8. Olivieri, A.; Fazzini, C.; Medda, E. Multiple Factors Influencing the Incidence of Congenital Hypothyroidism Detected by Neonatal Screening. *Horm. Res. Paediatr.* **2015**, *83*, 86–93. [CrossRef]
9. Larson, C.; Hermos, R.; Delaney, A.; Daley, D.; Mitchell, M. Risk Factors Associated with Delayed Thyrotropin Elevations in Congenital Hypothyroidism. *J. Pediatr.* **2003**, *143*, 587–591. [CrossRef]
10. Seetho, S.; Kongwattanakul, K.; Saksiriwuttho, P.; Thepsuthammarat, K. Epidemiology and Factors Associated with Preterm Births in Multiple Pregnancy: A Retrospective Cohort Study. *BMC Pregnancy Childbirth* **2023**, *23*, 872. [CrossRef]
11. Delange, F. Screening for Congenital Hypothyroidism Used as an Indicator of the Degree of Iodine Deficiency and of Its Control. *Thyroid* **1998**, *8*, 1185–1192. [CrossRef]
12. Hinton, C.F.; Harris, K.B.; Borgfeld, L.; Drummond-Borg, M.; Eaton, R.; Lorey, F.; Therrell, B.L.; Wallace, J.; Pass, K.A. Trends in Incidence Rates of Congenital Hypothyroidism Related to Select Demographic Factors: Data from the United States, California, Massachusetts, New York, and Texas. *Pediatrics* **2010**, *125*, S37–S47. [CrossRef]
13. Cornejo, V.; Raimann, E.; Cabello, J.F.; Valiente, A.; Becerra, C.; Opazo, M.; Colombo, M. Past, Present and Future of Newborn Screening in Chile. *J. Inherit. Metab. Dis.* **2010**, *33*, 301–306. [CrossRef]
14. Grob, F.; Bedregal, P.; Viviani, P.; Torres, C.; Becerra, C.; Leyton, B. Cognitive Outcomes in Congenital Hypothyroidism: Impact of Socioeconomic Status—A Case-Control Study. *Horm. Res. Paediatr.* **2022**, *95*, 224–232. [CrossRef]

15. McGrath, N.; Hawkes, C.P.; McDonnell, C.M.; Cody, D.; O'Connell, S.M.; Mayne, P.D.; Murphy, N.P. Incidence of Congenital Hypothyroidism Over 37 Years in Ireland. *Pediatrics* **2018**, *142*, e20181199. [CrossRef]
16. Yao, Y.; Deng, K.; Zhu, J.; Xiang, L.; Yuan, X.; Li, Q.; Liu, L.; Xu, W. Increased Incidence of Congenital Hypothyroidism in China: An Analysis of 119 Million Screened Newborns. *Eur. J. Pediatr.* **2023**, *182*, 4477–4486. [CrossRef]
17. Darrat, M.; Kayes, L.; Woodside, J.V.; Mullan, K.; Abid, N. Congenital Hypothyroidism in Northern Ireland: 40 Years' Experience of National Screening Programme. *Clin. Endocrinol.* **2023**, *99*, 409–416. [CrossRef]
18. Chamot, S.; Al-Salameh, A.; Balcaen, T.; Petit, P.; Bonneterre, V.; Cancé, C.; Desailloud, R. Congenital and Acquired Hypothyroidism: Temporal and Spatial Trends in France from 2014 to 2019. *Ann. Epidemiol.* **2024**, *98*, 18–24. [CrossRef]
19. Danner, E.; Niuro, L.; Huopio, H.; Niinikoski, H.; Viikari, L.; Kero, J.; Jääskeläinen, J. Incidence of Primary Congenital Hypothyroidism over 24 Years in Finland. *Pediatr. Res.* **2023**, *93*, 649–653. [CrossRef]
20. Albert, B.B.; Cutfield, W.S.; Webster, D.; Carll, J.; Derraik, J.G.B.; Jefferies, C.; Gunn, A.J.; Hofman, P.L. Etiology of Increasing Incidence of Congenital Hypothyroidism in New Zealand from 1993–2010. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 3155–3160. [CrossRef]
21. Lain, S.; Trumpff, C.; Grosse, S.D.; Olivieri, A.; Van Vliet, G. Are Lower TSH Cutoffs in Neonatal Screening for Congenital Hypothyroidism Warranted? *Eur. J. Endocrinol.* **2017**, *177*, D1–D12. [CrossRef]
22. Grob, F. Approaching the Diagnosis of Thyroid Disorders in Preterm Infants. *Pediatr. Res.* **2022**, *91*, 1021–1022. [CrossRef]
23. Eugène, D.; Djemli, A.; Van Vliet, G. Sexual Dimorphism of Thyroid Function in Newborns with Congenital Hypothyroidism. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 2696–2700. [CrossRef]
24. McNally, R.J.Q.; Jones, J.H.; Shaikh, M.G.; Donaldson, M.D.C.; Blakey, K.; Cheetham, T.D. Congenital Hypothyroidism: Space-Time Clustering of Thyroid Dysgenesis Indicates a Role for Environmental Factors in Disease Etiology. *Thyroid* **2021**, *31*, 876–883. [CrossRef]

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Experiences and Challenges with Congenital Hypothyroidism Newborn Screening in Indonesia: A National Cross-Sectional Survey

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Abstract: The expansion of newborn screening (NBS) for congenital hypothyroidism (CH) is essential to reducing the number of preventable intellectual disabilities in children. Because of logistical issues, including geographic extremes, distinct cultures, and 4.8 million births annually, Indonesia has struggled to achieve universal NBS coverage. A national cross-sectional electronic survey was conducted to explore challenges in CH NBS. Responses from 423 healthcare professionals and program administrators across 30 provinces in Indonesia were collected. The major challenges reported were refusal from families (39.2%), newborns being discharged <24 h (38.3%), and limited availability of filter paper (35.9%). The respondents considered refusal from families to be due to fear, while others did not understand the necessity of CH NBS. The vast majority of respondents believed that parents do not have sufficient understanding regarding CH NBS (96.5%). Our study found that only 38.5% of respondents had received formal CH NBS training, with pediatric endocrinologists being the only profession in which all respondents had been trained. Concerted efforts are needed to improve the access to and availability of resources, increase the capacity for sample collection and analysis, empower healthcare professionals, and develop educational resources to promote understanding and acceptance of NBS amongst families.

Keywords: newborn screening; congenital hypothyroidism; experiences; challenges

1. Introduction

With an estimated global incidence ranging from 1:2000 to 1:4000 live births, congenital hypothyroidism (CH) is the most common preventable cause of intellectual disability [1–4]. Early identification and treatment of affected newborns through national, whole-population screening has been shown to be effective in preventing developmental sequelae. Newborn screening (NBS) has been hailed as one of the greatest public health successes globally. CH NBS was first developed in 1972 in Quebec, Canada, by Professor Jean Dussault [5]. Since then, most high-income countries have successfully implemented NBS programs. However, the same cannot be said for a large number of low- and middle-income countries (LMICs), where the majority of newborns are not screened for CH [6,7]. The introduction and continuing expansion of NBS across the world is a critical step to achieving the Sustainable

Development Goals Nos. 3.2 and 3.4 to reduce the premature morbidity and mortality of neonates due to non-communicable diseases [8].

Indonesia is the world's largest archipelago, the fourth-most populated country, and has the fifth-most births internationally, with 4.8 million births annually [9]. In May 1999, eleven Southeast Asian countries, including Indonesia, convened for the Workshop on National Neonatal Screening for CH, where a consensus was made to initiate and develop regional CH NBS programs [10]. With the support of the International Atomic Energy Agency (IAEA), Indonesia began a pilot study involving Dr. Cipto Mangunkusumo Hospital in Jakarta and Dr. Hasan Sadikin Hospital in Bandung from 2000 to 2005 [11,12]. Upon the completion of a health technology assessment (HTA) in 2006, the CH NBS program expanded to eight provinces in 2008 (West Sumatra, Jakarta, West Java, Central Java, East Java, Yogyakarta, Bali, and South Sulawesi), and in 2014, the Ministry of Health (MOH) released a decree mandating CH NBS [13]. While CH NBS has subsequently continued to expand to other provinces, national CH NBS coverage has remained low and was reported to be 2.3% in 2022 [14]. This prompted the MOH to relaunch CH NBS in August 2022 [15], followed by the release of a national policy that makes CH NBS a requirement for all healthcare professionals to claim delivery payments from the national insurance in September 2023 [16].

Although most high-income countries have achieved universal screening coverage, Indonesia is still working towards developing a cost-effective, efficient approach as well as increasing awareness among the general public and healthcare professionals regarding NBS. The aim of this study was to identify barriers preventing the wider uptake of NBS in Indonesia. To achieve this, a national, cross-sectional online survey was conducted by Global Pediatric Endocrinology and Diabetes (GPED, www.globalpedendo.org [accessed on 2 December 2023]) and the Endocrinology Working Group of the Indonesian Pediatric Society (IPS). The focus was on identifying and exploring the experiences of healthcare professionals and program administrators in CH NBS. This is the first national survey to be conducted on CH NBS in Indonesia with respondents directly involved in the national program's implementation.

2. Materials and Methods

The survey was conducted in conjunction with a national webinar hosted by GPED, a non-profit organization with a focus on improving the care of children with endocrine disorders in LMICs, and the Endocrinology Working Group of IPS. All health professionals self-registering as interested by neonatal congenital hypothyroidism screening in Indonesia could attend this free webinar. However, the organizers actively reached out to members of IPS, the Indonesian Midwives Association and MOH public health office networks for the promotion of this webinar. Survey questions were disseminated at the end of the webinar on 3 September 2023. Healthcare professionals (n = 957) joined via Zoom and YouTube Live. Webinar participants who consented to the survey provided responses. Google Forms, a web-based survey software, was used to collect and store responses in a digital spreadsheet.

The survey consisted of four main sections, beginning with an informed consent section that detailed the objectives of the survey. After consent was received, respondents were directed to the next section, which comprised five questions on their demographic background. The final question in this section screened for webinar participants who worked in health facilities that provide CH NBS. Those who confirmed that they worked in health facilities that conduct CH NBS were eligible to proceed with the survey questions described in the third section. This third section consisted of seven questions on the challenges of CH NBS experienced by each respondent in their respective health facilities. The fourth and final section of the survey explored the attitudes of respondents towards CH NBS through eight "yes" or "no" questions. The survey took approximately 5 to 10 min to complete.

The subjects included in this study were healthcare professionals and administrative staff of public health offices working in health facilities directly involved in the CH

NBS program across Indonesia. During the data analysis process, duplicate responses were removed and descriptive statistics were used. Responses to open-ended questions were summarized by approximating the similarities in semantic content. The answers to the survey questions were treated anonymously. Ethics approval was not required for this survey.

3. Results

3.1. Respondent Demographics

A total of 481 responses were gathered in the survey disseminated during the GPED-IPS joint webinar. After removing duplicate responses, a total of 423 responses were included in the final analysis. Consistent with a national webinar that included hundreds of participants, there was great diversity in terms of the demographic backgrounds of the respondents. The demographic profiles of the respondents are shown in Table 1.

Table 1. Respondent demographics.

Respondent Demographics	n (%)
Profession (n = 423)	
Midwives	122 (28.8)
General practitioners	97 (22.9)
Pediatricians	91 (21.5)
Pediatric endocrinologists	11 (2.6)
Clinical pathologists	25 (5.9)
Medical laboratory technologists	18 (4.3)
Nurses	37 (8.8)
Public health officers	5 (1.2)
Screening program administrators	6 (1.4)
Others	11 (2.6)
Health facility level (n = 423)	
Public health office	8 (1.9)
Referral laboratory	1 (0.2)
Primary health center	87 (20.6)
Type A hospital *	39 (9.2)
Type B hospital *	76 (18.0)
Type C hospital *	128 (30.3)
Type D hospital *	51 (12.1)
Private clinics/hospitals	33 (7.8)

* Type A hospitals are national referral hospitals that cater to between 13 and 20 subspecialties. Type B hospitals provide the services of 4 basic specialties, 4 medical support specialties, 8 other specialties, and 2 basic subspecialties. Type C hospitals have 4 basic specialties and 4 medical support specialties. Type D hospitals have 2 basic specialties.

There were over a dozen different professions involved in our study, with the majority of participants being split between midwives (28.8%), general practitioners (22.9%), and pediatricians (21.5%). Besides clinicians involved in NBS, our survey also attracted professions involved in the sample analysis and public health aspect of NBS. Our study included representatives from each health facility level in Indonesia, with most participants working at type C hospitals (30.3%) and primary health centers (20.6%). Finally, out of 38 provinces in Indonesia, there were representatives from 30. Most respondents were from the Java region, notably East Java, West Java, and Central Java (23.4%, 13.2%, and 11.8%, respectively). Other responses were spread across other provinces located on the major islands, such as Kalimantan, Sumatera, Sulawesi, and Papua.

3.2. Implementation of Newborn Screening for Congenital Hypothyroidism

Table 2 summarizes the current state of CH NBS implementation among the facilities represented by the respondents. Most respondents reported that NBS for CH had been initiated in their respective health facilities over the last 3 years. One third of respondents indicated that their healthcare facility has not been able to screen all newborns, primarily because of insufficient availability of filter paper (33.1%), refusal from families (19.8%), or the NBS not being viewed as a compulsory national program before September 2023 (15.4%).

Table 2. Implementation of congenital hypothyroidism newborn screening.

Responses	n (%)
Since when has your health facility implemented the CH NBS policy? (n = 423)	
2000–2010	6 (1.4)
2011–2020	61 (14.4)
2021–2022	55 (13.0)
2023	169 (40.0)
Unsure/unaware	132 (31.2)
Are all babies born in your health facility screened? (n = 423)	
Yes	287 (67.8)
No	136 (32.2)
If you answered no to the previous question, provide your reason… (n = 136)	
Limited stock of filter paper	45 (33.1)
Refusal from families	27 (19.8)
NBS was not compulsory previously	21 (15.4)
Healthy infants discharged <24 h post birth	14 (10.3)
Critically ill newborns	10 (7.4)
Lack of trained healthcare professionals for sample collection	6 (4.4)
Only patients with ID cards in the respective cities can be screened	5 (3.7)
Infants without national health insurance	4 (2.9)
Sample delivery problems	1 (0.7)
No reason provided	3 (2.2)

3.3. Challenges in Congenital Hypothyroidism Newborn Screening

3.3.1. General Challenges in National Program Implementation

The challenges uncovered in our study are summarized in Table 3. The three major challenges reported by respondents were refusal from families (39.2%), sampling time difficulty as newborns were discharged at <24 h of age (38.3%), and limited availability of filter paper (35.9%). In addition to NBS, our study explored whether levothyroxine was available for CH management. There were a concerning large number of responses indicating that levothyroxine was not available (31.2%), while 43.3% reported that it was consistently available.

Table 3. Challenges in CH NBS implementation.

Responses	n (%)
Have you experienced any of the following difficulties in implementing CH NBS? *	
Parents who refuse screening	166 (39.2)
Sampling time is difficult because infants are often discharged <24 h	162 (38.3)
Limited availability of filter paper	152 (35.9)
Technical difficulties in sample collection	107 (25.3)
Difficulties in sending samples to referral labs	73 (17.3)
Difficulty with re-screening/confirmation tests on positive screening results	49 (11.6)
Financial barriers to confirmation tests	63 (14.9)
Others:	
Results of screening that take a long time	5 (1.2)
Lack of trained healthcare professionals for sample collection	5 (1.2)
Financial challenges (non-NHI patients, OOP when filter papers are unavailable) **	4 (1.0)
Geographical barriers	2 (0.5)
Confusion on whether to screen in special cases (sick infants, premature infants, etc.)	1 (0.2)
Lack of collaboration with referral labs	1 (0.2)
Patients who do not come to the health facility for their scheduled screening	1 (0.2)
Have not experienced difficulty	25 (5.9)
Is levothyroxine available for CH management in your health facility? (n = 423)	
Yes	183 (43.3)
No	132 (31.2)
Unaware/unsure	108 (25.5)

* Respondents were allowed to select multiple responses. ** NHI: national health insurance, OOP: out-of-pocket.

3.3.2. Challenges from the Parents' Perspectives

Our study explored the reasons behind parent's refusal through the perspectives of healthcare professionals (Table 4). Most respondents reported that one of the reasons for parental refusal is the fear of seeing their newborn having blood drawn from their heel (49.4%), while others do not understand the necessity of CH NBS (31%). There are also general fears regarding the CH NBS procedure and potential side effects (32.2%). There were several respondents who reported never encountering parents who refused screening (15.6%).

Our survey also explored the healthcare professionals' experience with parents' education levels in the field (Table 5). Almost all respondents shared their belief the parents do not have sufficient knowledge and understanding regarding CH NBS (96.5%). When asked regarding platforms to educate parents about CH NBS, most participants agreed that this should be incorporated into routine antenatal care (53.2%). Others supported the idea of educational content through social media (20.8%) and community health center outreach activities (17.3%).

Table 4. Refusal of CH NBS screening by Indonesian parents.

Responses	n (%)
If you have ever encountered parents who refused screening, what were their reasons for refusing to be screened? *	
Can't bear to see their child having blood drawn on the heel	209 (49.4)
Afraid	136 (32.2)
Don't feel the need	131 (31.0)
Hearing fake news related to NBS	45 (10.6)
Religious beliefs	43 (10.2)
Additional financial expenditure (non-NHI patients, filter papers unavailable) **	5 (1.2)
Healthcare professionals who were unable to provide adequate and clear explanations regarding NBS and its benefits	2 (0.5)
Have never met parents who refused screening	66 (15.6)

* Respondents were allowed to select multiple responses. ** NHI: national health insurance.

Table 5. Indonesian healthcare professionals' perspectives towards parental understanding.

Responses	n (%)
Do you think parents in Indonesia have sufficient knowledge regarding CH NBS? (n = 423)	
Yes	15 (3.5)
No	408 (96.5)
If you answered "No" to the previous question, what form of education do you think is effective for parents in Indonesia? *	
Education during routine antenatal care	225 (53.2)
Educational content through social media	88 (20.8)
Community health center outreach activities	73 (17.3)
Educational material in a book/booklet	17 (4.0)
Door to door education	10 (2.4)
Online seminars	6 (1.4)
Offline seminars	3 (0.7)
Revise the national maternal and child health book	1 (0.2)

* Respondents were allowed to select multiple responses.

3.3.3. Challenges from the Perspectives of Healthcare Professionals

Challenges highlighted by healthcare professionals conducting NBS mainly revolved around a lack of training and technical skills in sample collection. Our study found that only 38.5% of respondents had received formal CH NBS training (Table 6). Only 23.7% to 66.7% of the respondents within each group of profession had received training regarding CH NBS. Pediatric endocrinologists were the only profession reported in our study where all respondents had been trained specifically regarding CH NBS. This result was independent from the type of healthcare facility. For public healthcare facilities, 147 respondents (37.7%) stated that they have received CH NBS training while 243 respondents (62.3%) have not. For private healthcare facilities, 16 respondents stated they have received training (48.5%), while 17 (51.5%) have not (chi-square test 1.50, $p = 0.22$). The large majority of respondents echoed the need for additional training regarding NBS (93.6%).

Table 6. Training of healthcare professionals on congenital hypothyroidism newborn screening.

Responses	n (%)
Have you ever received formal training regarding CH NBS? (n = 423)	
Yes	163 (38.5)
No	260 (61.5)
Respondents who have received training based on healthcare profession	
Midwives	42/122 (34.4)
General practitioners	23/97 (23.7)
Pediatricians	51/91 (56.0)
Pediatric endocrinologists	11/11 (100)
Nurses	11/37 (29.7)
Clinical pathologists	12/25 (48.0)
Medical laboratory technologists	5/18 (27.8)
Public health officers	2/5 (40.0)
Screening program administrators	4/6 (66.7)
Do you feel you need additional training regarding CH NBS? (n = 423)	
Yes	396 (93.6)
No	27 (6.4)
If yes, what form should this training take place? *	
Offline seminars	169 (42.7)
Online seminars	130 (32.8)
Educational material in a book/booklet	87 (22.0)
Educational content through social media	17 (4.3)
Hands on workshop	14 (3.5)

* Respondents were allowed to select multiple responses.

3.4. Attitudes towards Congenital Hypothyroidism Newborn Screening

The final section of our survey explored the attitudes of healthcare professionals and staff involved in CH NBS towards CH NBS itself (Table 7). The vast majority of respondents had positive attitudes towards CH NBS. While most respondents felt confident enough to educate and answer parents' questions regarding NBS (87%), fewer felt that they had sufficient understanding regarding the CH NBS system in Indonesia (69.5%).

Table 7. Attitudes towards congenital hypothyroidism newborn screening.

Responses	n (%)
Does CH NBS add additional burden to your work? (n = 423)	
Yes	55 (13.0)
No	368 (87.0)
Does CH NBS provide benefits in improving children's health? (n = 423)	
Yes	421 (99.5)
No	2 (0.5)
Have you received sufficient training related to CH NBS? (n = 423)	
Yes	197 (46.6)
No	226 (53.4)
Do you feel confident enough to educate and answer parents' questions regarding CH NBS? (n = 423)	
Yes	368 (87.0)
No	55 (13.0)
Do you feel like you have sufficient understanding about the CH NBS in Indonesia, including the referral system, algorithm of positive results, etc.? (n = 423)	
Yes	294 (69.5)
No	129 (30.5)
Should all newborns be screened? (n = 423)	
Yes	406 (96.0)
No	17 (4.0)
Is newborn screening the responsibility of all health workers? (n = 423)	
Yes	399 (94.3)
No	24 (5.7)
Should Indonesia conduct screening for other congenital diseases such as congenital adrenal hyperplasia (CAH)? (n = 423)	
Yes	398 (94.1)
No	25 (5.9)

4. Discussion

With 423 responses from ten different professional backgrounds spread across 30 provinces, the findings of this study highlight the specific challenges still facing CH NBS implementation in Indonesia. Despite being initiated over two decades ago, the national coverage of CH NBS is still low, with data from 2022 revealing a coverage of only 2.3% of all newborns. Encouragingly, the country has seen a significant increase in government commitment and support for the CH NBS national program. Unpublished data from the MOH show that the number of samples increased steadily in 2023, standing at 48,887 samples a week in the third week of October 2023. This brings the national report of CH NBS samples collected in 2023 to 692,744 as of the third week of October 2023, translating to a national coverage of 15.53% [16]. However, current reports show that there is still great disparity between provinces, with reported coverage from as low as 0.02% in South Papua to 40.54% in Bali [16].

CH NBS in Indonesia has progressed from being the subject of a pilot study involving two hospitals to being implemented across 38 provinces. Without laboratories specifically

designated for newborn screening, our CH NBS program relies on referral laboratories appointed by the MOH. Public health centers, hospitals, and other healthcare facilities that conduct NBS send samples to their appointed referral laboratory for the initial screening of CH. Positive results are then reported back to the healthcare facility of origin to arrange further tests as needed. While several referral laboratories have been assigned since 2008, our study reveals that the majority of healthcare facilities only started screening for CH in 2023 (40%), with many reporting that the recent MOH policy making CH NBS compulsory to claim newborn delivery payments from the national insurance acted as the catalyst for CH NBS initiation. Very few respondents (1.4%) reported that the implementation began within the first decade the program was introduced in Indonesia.

The implementation of a national NBS program is complex, with the involvement of multiple stakeholders, and comprises several areas, including education, screening, follow-up of results, diagnosis, management, and evaluation, all of which must be institutionalized within national public health systems. In developing countries, especially in the Southeast Asian and North African regions, the initiation and implementation of NBS programs are limited by a number of challenges from varying public health priorities, such as low socio-economic status and poor parental awareness [10,17,18]. Our study found that Indonesia is also still limited by these challenges.

One of the most common problems mentioned by respondents was difficulty with sampling time, as patients tended to be discharged less than 24 h after birth. Studies conducted in Sri Lanka and Malaysia have also found this to be a common hurdle [19,20]. The American Academy of Pediatrics recommends that the optimal time for testing is between 48 and 72 h of age [21]. With the initial TSH surge post-birth, specimens collected within the first 24 h are associated with higher false positive rates [22]. From a cost–benefit perspective, high false positive rates are unfavorable due to negative psychological effects and stress on families, as well as the increased costs associated with confirmation tests [23,24]. In Indonesia, while the costs of the initial CH NBS are covered by the MOH, currently, not all confirmation tests for patients are covered. Patients must either arrange for further testing in the referral laboratories located in major cities or pay out-of-pocket with other providers. A solution often suggested by experts is to introduce higher cutoffs for specimens collected <24 h of age. However, it must be noted that introducing higher cutoffs comes with the risk of missing a true case of CH [25].

In addition to early discharge of patients, Indonesia faces added challenges such as the fact that not all births take place in healthcare facilities and not all families have general care physicians that provide long-term care. Most antenatal and postpartum care of mothers and children in the country is provided by midwives. A study by Efendi et al. found that only 55.2% of Indonesian mothers seek birth assistance from healthcare providers [26]. Sri Lanka is another Southeast Asian country that started CH NBS programs within the last decade, and almost 99% coverage was achieved within four months of its implementation [20]. Such success was possible as the vast majority of births took place in hospitals or in maternity homes. Similarly, high-income countries, where births generally take place in maternity units, have also reported coverage exceeding 99.5% [27]. Learning from the experiences of other countries where universal coverage for screening has been achieved, Indonesia needs to consider revising the current regulations and guidelines for CH NBS or making adjustments to national health insurance policies for neonatal and postpartum care. While changes are needed to improve NBS uptake, the major logistical issues associated with such a large newborn population and the distribution of births over many islands needs emphasizing. Indonesia has unique challenges, and these will take time to resolve.

Building a sustainable CH NBS program depends on the availability of robust infrastructure equipped with adequate logistical support. The findings of this study reveal that another major challenge in achieving universal screening coverage is the lack of basic consumables, such as the availability of NBS papers. At the time this study was conducted, Indonesia only had twelve designated national referral laboratories, limiting the number of samples that can be analyzed each week and further complicating the process with

lengthy processing and reporting times. The current national capacity for screening is still below the recommendations of the Working Group of Neonatal Screening of the European Society for Pediatric Endocrinology, which states that screening should be conducted in centralized laboratories that cover 100,000 newborns per year [28]. Even with the increasing government commitment and support, universal screening coverage cannot be achieved without adequate facilities and infrastructural support.

Parental understanding, perspectives, and concerns surrounding CH NBS is a critical factor that determines the success of a national NBS program. Parents need to be well-informed regarding CH and the benefits of NBS to provide the necessary consent, and this remains a challenge in Indonesia. A study by Biswas et al. on parental perception towards newborn metabolic screening in Indonesia found that, once parents understood the principles behind metabolic testing, they consented to screening [29]. While most respondents felt confident about educating parents (87%), they found that Indonesian parents do not have sufficient knowledge and understanding regarding CH NBS (96.5%). The American College of Obstetrics and Gynecology recommends that NBS information should be included during prenatal visits through the postpartum period, with the optimal time to provide NBS education being the third trimester of pregnancy [30]. As CH is the first disorder to be identified for a national NBS program in Indonesia, the public is largely unaware of the purpose and benefits of NBS. A study by Rama Devi et al. shared the experience of initiating an NBS program in a rural area of Andhra Pradesh, India, and reported that, with repeated awareness and training regarding NBS, the program was able to be a success despite initial struggles with the local population's illiteracy, ignorance, and taboos, as well as a lack of awareness and interest amongst the medical community [31]. Parental education regarding the importance and relevance of NBS should be incorporated across maternal and childcare services, including campaigns targeted to increase awareness of NBS, inclusion in the national mother and child health book, and a national consensus that ensures expecting parents are educated regarding NBS regardless of the individual providing their antenatal care.

Our survey was conducted alongside a national webinar hosted by GPED and the Endocrinology Working Group of IPS. Even though this study does not directly assess the level of knowledge of healthcare professionals in Indonesia, the questions submitted by our webinar participants were mainly basic questions surrounding CH as a disorder and the CH NBS concept, process, and benefits. We can see that healthcare professionals in Indonesia still lack the knowledge and understanding needed to educate parents to instill trust and elicit acceptance of CH NBS. Our study also found that pediatric endocrinologists are the only profession to receive formal CH NBS training. This prompted most respondents to express the need for additional training regarding NBS (93.6%). These training programs should include hands-on workshops targeted at healthcare professionals directly involved in sample collection, which, in our case, are mainly midwives. Finally, a socialization program regarding the CH NBS system also needs to be established, as only 69.5% of respondents felt that they had sufficient understanding regarding the CH NBS system in Indonesia.

While the initial focus of the NBS program will be on increasing screening coverage in Indonesia, the program's success also involves contacting and appropriately treating affected infants. Patients with positive tests must undergo confirmation tests, and ultimately, once diagnosed with CH, access to and availability of treatment must be ensured. While levothyroxine is widely available, as of 2023, there are only 39 pediatric endocrinologists nationally [32]. Therefore, currently, pediatric endocrinologists work with pediatricians to manage CH. As we gradually work towards universal screening for CH and anticipate more confirmed CH cases, adequate training and distribution of pediatricians capable of managing CH need to be established nationally.

As Indonesia continues to work towards improving national coverage for CH NBS and developing NBS for other disorders, such as congenital adrenal hyperplasia and G6PD, we hope that the findings of this study can provide preliminary data to help shape and

refine existing policies and programs to reach the ultimate goal of ensuring that every newborn in Indonesia is screened.

5. Limitations

Although we included representatives of over ten different professional backgrounds, additional perspectives are required, including those from parents and obstetricians. This study mainly reflects the experiences of healthcare professionals and staff involved in CH NBS in Indonesia, but does not objectively assess respondents' knowledge and attitudes. The vast majority of the respondents were from public healthcare facilities and may also play more than one role, for instance, both clinical and administrative. There was also a lack of representation from healthcare professionals working in private healthcare facilities. As CH screening improves, future surveys should take into account this limitation, provide the opportunity to list more than one role in the process of CH screening, and actively reach out to those working in private healthcare facilities. Lastly, further studies aiming to gather quantitative data regarding CH NBS in Indonesia should recruit more respondents to provide better national representation.

6. Conclusions

CH NBS in Indonesia faces challenges such as a lack of logistical and infrastructural support, lack of knowledge and training of healthcare professionals, and hesitation from families. With the complexity of developing and maintaining a national program of this scale, funding is not the only driver of success. Our study highlights that concerted efforts must be made to improve the access to and availability of resources, increase the capacity for sample collection and analysis, educate and empower healthcare professionals, and develop educational materials and programs to promote the understanding and acceptance of NBS amongst families.

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References

1. Mitchell, M.L.; Hsu, H.W.; Sahai, I. The increased incidence of congenital hypothyroidism: Fact or fancy? *Clin. Endocrinol.* **2011**, *75*, 806–810. [CrossRef] [PubMed]
2. Büyükgebiz, A.; Gunoz, H.; Kurtoglu, S. Congenital hypothyroidism clinical aspects and late consequences. *Pediatr. Endocrinol. Rev.* **2003**, *1* (Suppl. 2), 185–190. [PubMed]
3. Bongers-Schokking, J.J.; De Muinck Keizer-Schrama, S.M.P.F. Influence of timing and dose of thyroid hormone replacement on mental, psychomotor, and behavioral development in children with congenital hypothyroidism. *J. Pediatr.* **2005**, *147*, 768–774. [CrossRef] [PubMed]
4. Pulungan, A.B.; Oldenkamp, M.E.; van Trotsenburg, A.S.P.; Windarti, W.; Gunardi, H. Effect of delayed diagnosis and treatment of congenital hypothyroidism on intelligence and quality of life: An observational study. *Med. J. Indones.* **2019**, *28*, 396–401. [CrossRef]

5. Dussault, J.H. The anecdotal history of screening for congenital hypothyroidism. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 4332–4334. [CrossRef]
6. Kopel, J. A global perspective on newborn congenital hypothyroidism screening. *Baylor Univ. Med. Cent. Proc.* **2020**, *33*, 137–139. [CrossRef]
7. LaFranchi, S.H. Worldwide coverage of newborn screening for congenital hypothyroidism-a public health challenge. *US Endocrinol.* **2014**, *10*, 115–116. [CrossRef]
8. World Health Organization. Targets of Sustainable Development Goals 3. Available online: https://www.who.int/europe/about-us/our-work/sustainable-development-goals/targets-of-sustainable-development-goal-3 (accessed on 1 November 2023).
9. Badan Pusat Statistik. Population Projection by Province, 2010–2035 (Thousands). 2019. Available online: https://www.bps.go.id/publication/2013/10/07/053d25bed2e4d62aab3346ec/proyeksi-penduduk-indonesia-2010-2035.html (accessed on 24 September 2023).
10. Padilla, C.D.; Therrell, B.L. Newborn screening in the Asia Pacific region. *J. Inherit. Metab. Dis.* **2007**, *30*, 490–506. [CrossRef]
11. Pulungan, A.B.; Soesanti, F.; Utari, A.; Pritayati, N.; Julia, M.; Annisa, D.; Andarie, A.A.; Bikin, I.W. Preliminary study of newborn screening for congenital hypothyroidism and congenital adrenal hyperplasia in Indonesia. *eJournal Kedokt. Indones.* **2020**, *8*, 98–103.
12. Therrell, B.L.; Padilla, C. *Screening of Newborns for Congenital Hypothyroidism: Guidance for Developing Programmes*; International Atomy Energy Agency: Vienna, Austria, 2005.
13. Ministry of Health Republic of Indonesia. *Ministry of Health Republic of Indonesia Regulation No. 78 Year 2014 on Congenital Hypothyroidism Screening*; Ministry of Health Republic of Indonesia: Jakarta, Indonesia, 2014.
14. Ministry of Health Republic of Indonesia. *Indonesian Basic Health Research (RISKESDAS) 2018*; Ministry of Health Republic of Indonesia: Jakarta, Indonesia, 2018.
15. Kementerian Kesehatan Republik Indonesia. Kemenkes Relaunching Skrining Hipotiroid Kongenital Untuk Kurangi Risiko Kecacatan Pada Anak. 2022. Available online: https://sehatnegeriku.kemkes.go.id/baca/rilis-media/20220831/2641015/kemenkes-relaunching-skrining-hipotiroid-kongenital-untuk-kurangi-risiko-kecacatan-pada-anak/ (accessed on 24 September 2023).
16. Ministry of Health Republic of Indonesia. *Congenital Hypothyroidism Newborn Screening Coverage Report*; Ministry of Health Republic of Indonesia: Jakarta, Indonesia, 2023.
17. Padilla, C.D. Towards universal newborn screening in developing countries: Obstacles and the way forward. *Ann. Acad. Med. Singapore.* **2008**, *37* (Suppl. 12), 6–9.
18. Anne, R.P.; Rahiman, E.A. Congenital hypothyroidism in India: A systematic review and meta-analysis of prevalence, screen positivity rates, and etiology. *Lancet Reg. Health-Southeast Asia* **2022**, *5*, 100040. [CrossRef] [PubMed]
19. Rose, S.R.; Wassner, A.J.; Wintergerst, K.A.; Yayah-Jones, N.H.; Hopkin, R.J.; Chuang, J.; Smith, J.R.; Abell, K.; LaFranchi, S.H.; Section on Endocrinology Executive Committee; et al. Congenital hypothyroidism: Screening and management. *Pediatrics* **2023**, *151*, e2022060419. [CrossRef] [PubMed]
20. Leong, Y.H.; Gan, C.Y.; Tan, M.A.F.; Majid, M.I.A. Present status and future concerns of expanded newborn screening in Malaysia: Sustainability, challenges and perspectives. *Malays. J. Med. Sci.* **2014**, *21*, 63–67. [PubMed]
21. Hettiarachchi, M.; Amarasena, S. Indicators of newborn screening for congenital hypothyroidism in Sri Lanka: Program challenges and way forward. *BMC Health Serv. Res.* **2014**, *14*, 385. [CrossRef] [PubMed]
22. Lee, S.Y. Perinatal factors associated with neonatal thyroid-stimulating hormone in normal newborns. *Ann. Pediatr. Endocrinol. Metab.* **2016**, *21*, 206–211. [CrossRef] [PubMed]
23. Khairi, M.D.M.; Rafidah, K.N.; Affizal, A.; Normastura, A.R.; Suzana, M.; Normani, Z.M. Anxiety of the mothers with referred baby during Universal Newborn Hearing Screening. *Int. J. Pediatr. Otorhinolaryngol.* **2011**, *75*, 513–517. [CrossRef] [PubMed]
24. Mehran, L.; Khalili, D.; Yarahmadi, S.; Amouzegar, A.; Mojarrad, M.; Ajang, N.; Azizi, F. Worldwide recall rate in newborn screening programs for congenital hypothyroidism. *Int. J. Endocrinol. Metab.* **2017**, *15*, e55451. [CrossRef]
25. Lott, J.A.; Sardovia-Iyer, M.; Speakman, K.S.; Lee, K.K. Age-dependent cutoff values in screening newborns for hypothyroidism. *Clin. Biochem.* **2004**, *37*, 791–797. [CrossRef]
26. Efendi, F.; Ni'Mah, A.R.; Hadisuyatmana, S.; Kuswanto, H.; Lindayani, L.; Berliana, S.M. Determinants of facility-based childbirth in Indonesia. *Sci. World J.* **2019**, *2019*, 9694602. [CrossRef]
27. Pollitt, R.J.; Green, A.; McCabe, C.J.; Booth, A.; Cooper, N.J.; Leonard, J.V.; Nicholl, J.; Nicholson, P.; Tunaley, J.R.; Virdi, N.K. Neonatal screening for inborn errors of metabolism: Cost, yield and outcome. *Health Technol. Assess.* **1997**, *1*, 1–202. [CrossRef]
28. Toublanc, J.E.; Grüters-Kieslich, A.; Donaldson, M.; Ferrandez-Longas, A.; Giovannelli, G.; Honour, J.W.; Hnikova, O.; Klett, M.; Larsson, A.; De Muinck Keizer-Schrama, S.M.P.F.; et al. Revised guidelines for neonatal screening programmes for primary congenital hypothyroidism. *Horm. Res.* **1999**, *52*, 49–52.
29. Biswas, A.; Lee, L.; Joseph, R.; Stella, M.; Chan, Y. Parental perceptions: Effect on newborn metabolic screening uptake. *Ann. Acad. Med. Singap.* **2010**, *39* (Suppl.), S248.
30. ACOG committee opinion No. 778: Newborn screening and the role of the obstetrician-gynecologist. *Obstet Gynecol.* **2019**, *133*, e357–e361. [CrossRef] [PubMed]
31. Radha Rama Devi, A.; Ananthalakshmi, Y.; Srimannarayana Rao, K. Challenges and opportunities in establishing and maintaining newborn screening in a rural area of Andhra Pradesh—Task Force Study by Indian Council of Medical Research. *Indian. J. Pediatr.* **2018**, *85*, 948–951. [CrossRef] [PubMed]

32. Indonesian Pediatric Society. *Database of the Endocrinology Working Group*; Indonesian Pediatric Society: Jakarta, Indonesia, 2023.

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Article

Evaluation of the First Three Years of Treatment of Children with Congenital Hypothyroidism Identified through the Alberta Newborn Screening Program

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Abstract: The effectiveness of newborn screening (NBS) for congenital hypothyroidism (CH) relies on timely screening, confirmation of diagnosis, and initiation and ongoing monitoring of treatment. The objective of this study was to ascertain the extent to which infants with CH have received timely and appropriate management within the first 3 years of life, following diagnosis through NBS in Alberta, Canada. Deidentified laboratory data were extracted between 1 April 2014 and 31 March 2019 from Alberta Health administrative databases for infants born in this time frame. Time to lab collection was anchored from date of birth. Timeliness was assessed as the frequency of monitoring of Thyroid Stimulating Hormone (TSH) and appropriateness as the frequency of children maintaining biochemical euthyroidism. Among 160 term infants, 95% had confirmation of diagnosis by 16 days of age. The cohort had a median of 2 (range 0–5) TSH measurements performed in the time interval from 0 to 1 month, 4 (0–12) from 1 to 6 months, 2 (0–10) from 6 to 12 months, and 7 (0–21) from 12 to 36 months. Approximately half were still biochemically hypothyroid (TSH > 7 mU/L) at 1 month of age. After becoming euthyroid, at least some period of hypo- (60%) or hyperthyroidism (TSH < 0.2 mU/L) (39%) was experienced. More work needs to be performed to discern factors contributing to prolonged periods of hypothyroidism or infrequent lab monitoring.

Keywords: neonatal screening; congenital hypothyroidism; neurodevelopment; euthyroid; guidelines

1. Introduction

Thyroid hormone is essential to the developing brain and nervous system, especially before 3 years of age. Congenital hypothyroidism (CH) is a condition that describes an infant who is born without the ability to make sufficient thyroid hormone, most commonly due to a structurally abnormal gland or one that cannot make thyroid hormone. The diagnosis of primary CH is made by demonstrating elevated Thyroid Stimulating Hormone (TSH) and reduced free thyroxine (FT4) in serum.

Infants with CH may not display any obvious clinical symptoms during the neonatal period, making it difficult to diagnose. Delays in treatment may result in permanent intellectual disability. Early detection through newborn screening (NBS) and timely initiation of levothyroxine therapy successfully prevent intellectual disability and other negative consequences of untreated hypothyroidism in the affected neonates. The effectiveness of

newborn screening for CH in reducing the prevalence of intellectual disability has been incontrovertibly demonstrated [1,2].

In Canada, Quebec was the first province to provide universal newborn screening for CH in April 1974 [3]. In Alberta, this became available in 1977 [4]. The incidence of CH in Alberta has been reported to be approximately 1 in 3500, with 10 to 15 cases detected each year [5].

To be effective at preventing intellectual disability, positive screening results must be followed up appropriately and in a timely manner. A positive newborn screen for CH is confirmed with measurements of serum TSH and FT4 levels. Recommendations from American and European bodies suggest that levothyroxine therapy should be initiated as early as possible and by 14–15 days of age [6–9]. The goal of therapy is to reach euthyroidism with a normalized FT4 within two weeks and a normal TSH within one month of initiating treatment [6]. There is currently no formal Canadian guideline. Afterwards, the child generally remains on thyroid hormone replacement for at least 2 years and regularly undergoes blood testing to monitor the adequacy of treatment. Persistent hypothyroidism may lead to intellectual disability, while hyperthyroidism can result in adverse effects such as poor weight gain and premature craniosynostosis [10–12].

A previous study of the Alberta Newborn Screening Program (ANSP) for CH revealed heterogeneity of practice in diagnosis and proximal management, subsequent to notification of a positive CH screen [13], but there have been no studies detailing the outcomes of long-term monitoring over the subsequent 3 years. The objective of this study was to evaluate the extent to which infants with CH have received timely and appropriate management within the first 3 years of life subsequent to their identification through the ANSP.

2. Materials and Methods

Study design: This is a retrospective chart review of Alberta infants who screened positive for and were diagnosed with CH through the ANSP. Deidentified laboratory data were extracted from Alberta Health administrative databases for infants born between 1 April 2014 and 31 March 2019: Ambulatory Care, Inpatient, Laboratory, Pharmaceutical Information, and Population Registry. All outputs were limited to 0 to 3 years of age. For example, if the service event occurred on or after the recipient's 3rd-year birthday, the record was not included during the data extraction. All confirmed cases of CH identified for infants born during this time frame were included in the initial analyses of incidence and time to first confirmatory TSH measurement.

NBS for CH in Alberta: The ANSP is a province-wide screening program offered to all infants born in Alberta and is located at the University of Alberta Hospital in the provincial capital, Edmonton. The ANSP screens for 22 conditions, including congenital hypothyroidism due to primary causes. The NBS for CH is performed by measuring TSH levels from capillary dried blood specimens (DBSs) collected on Whatman 903 filter paper. Collection of the DBS is recommended at 24 to 72 h of age, as previously described [4]. During the period of this study, the TSH eluted from a single 3.2 mm DBS was analyzed through AutoDELFIA® Neonatal hTSH time-resolved fluoroimmunoassay (PerkinElmer, Turku, Finland), using AutoDELFIA instruments with the corresponding software (Multicalc 2.1 Rev3, PerkinElmer, Turku, Finland), in full compliance with the manufacturer's instructions.

The CH screening algorithm has two types of out-of-range results: borderline or critical. The borderline cutoff is age-related (0–30 h, TSH $\geq$ 32 mU/L; 31–191 h, TSH $\geq$ 26 mU/L; and $\geq$192 h, TSH $\geq$ 20 mU/L). The critical cutoff (TSH $\geq$ 50 mU/L) is the same for all age groups. Infants with borderline results on the initial specimen are followed up with a second NBS collection, whereas infants with critical results are immediately referred to a pediatric endocrinology specialist for a confirmatory diagnostic evaluation. Two borderline results (on separate specimens) are followed up as a critical result. Since CH may be missed in infants with low birth weight (BW) due to delayed TSH rise if screened early after birth, all infants with BW < 2000 g are recommended to have blood spots recollected at 21–28 days of age, even if the initial screen result was normal [4].

Timeliness of diagnosis: Timeliness of diagnosis was described as the time from birth to the date of confirmatory laboratory investigation of serum TSH and FT4. The most sensitive test for detecting primary CH is measurement of TSH [14]. Therefore, we focused our analyses on frequency and levels of TSH measurements.

Timeliness of monitoring: Timeliness of monitoring was assessed as the frequency of monitoring of thyroid function indices. It was anchored to the date of birth of the infant, rather than to the date of the abnormal NBS, because recommendations for treatment initiation and monitoring are expressed in terms of the infant's age. In 2013, the ANSP (then known as the Alberta Newborn Metabolic Screening Program) developed the "Clinical Algorithm for CH Abnormal Screen Result", which was based on recommendations from the American Academy of Pediatrics, the Lawson Wilkins Pediatric Endocrine Society (now known as the Pediatric Endocrine Society), and the American Thyroid Association [6,15,16]. TSH +/− FT4 levels should be monitored as follows:

At 2 weeks and then 4 weeks from 0 to 1 month old ($\geq$2 times);

Every 1 to 2 months for infants 1 to 6 months old ($\geq$3 times);

Every 3 to 4 months for infants 6 to 36 months ($\geq$7 times).

Appropriateness of treatment: The goals are to normalize serum FT4 levels within 2 weeks of age and serum TSH levels within 1 month of age. Once FT4 levels are normal, TSH should be used to adjust doses, aiming for TSH levels in the lower half of the normal range. The Alberta Clinical Algorithm recommends levels between 0.5 and 2 mU/L by one month of age and thereafter [16]. Infants born between 1 January and 31 March 2019 were not included in this portion of the analysis because there were insufficient data.

On 12 April 2016, the reference intervals for TSH and FT4 were aligned throughout Alberta according to the performing methodology [17]. Because of the immediate postnatal TSH surge, TSH and thyroid hormones are higher in infants and subsequently fall, approximating adult levels by about 1 month of age [18]. Before this alignment, some regional labs used an upper-limit TSH of 6.8 for infants 8 days to 1 year and 6.5 mU/L for age $\geq$ 1 year. The lower limit of the normal range of TSH was 0.2 mU/L. For our analyses, we defined biochemical hypothyroidism as TSH > 7 and hyperthyroidism as TSH < 0.2 mU/L. Subclinical hypothyroidism or hyperthyroidism occurs when the TSH level is increased or decreased, respectively, above the normal range, but the FT4 level is normal.

Premature infants can have a blunted or delayed TSH surge due to immaturity of their hypothalamic-pituitary-thyroidal axis [19]. In all premature infants with BW < 2000 g, their NBS are repeated between 21 and 28 days of age if the initial screen was normal. We include all confirmed cases of CH, including premature infants, when reporting the incidence of confirmed CH during the specified time frame. Subsequently, we excluded premature infants from further analyses because their timing and frequency of monitoring would skew the overall time-based results. (In addition, these infants were cared for in Neonatal Intensive Care Units, which is not reflective of most infants with CH who are monitored in the outpatient, community setting).

Statistical analysis: All statistical analyses were performed using SAS Ver. 9.4 and R Ver. 4.1.1. We computed the mode and the median because we were interested in both the most common values and the middle values. Categorical variables are presented as frequencies and percentages. Stratification variables include year of birth and infant age (in days) where appropriate.

3. Results

3.1. Timeliness of Diagnosis

Between 1 April 2014 and 31 March 2019, 273,319 infants were registered in Alberta, and out of these, 271,826 received NBS. Out-of-range (i.e., positive) NBS results for CH were reported for 247 babies. Further diagnostic testing revealed 185 true-positive NBS results for CH, an incidence of 1:1470.

Infants most commonly were 6 or 7 days of age at the time of the first confirmatory TSH measurement. Most infants (84%) had biochemical confirmation of diagnosis within 16 days of age (range 1 to 69 days). Among cases where borderline NBS results required a second or third repeat screening, confirmation of hypothyroidism by venous sampling typically occurred within one week from when the overtly positive NBS was reported.

When premature infants or infants who died were removed from the analysis, there were 160 infants who had confirmation of positive screens by 2 to 45 days, with 95% of infants being diagnosed within 16 days of age (Table 1 and Figure 1). In 4 cases, a venous FT4 was collected (not TSH) before day 6, and the results were sufficiently low to prompt treatment. (TSH levels were done afterwards and remained high, verifying CH due to primary causes.) In addition, there was one infant with several equivocal NBS with first diagnostic TSH at day 28, one infant with a positive NBS confirmed one month later on day 45, and two infants who did not have diagnostic testing until days 17 and 19 for unknown reasons.

Table 1. Age of infant in postnatal days at the time of first venous TSH measurement.

Year	2014 (*n* = 27)	2015 (*n* = 35)	2016 (*n* = 30)	2017 (*n* = 39)	2018 (*n* = 25)	2019 (*n* = 4)
			Time to 1st TSH (Days)			
Range	5 to 16	2 to 28	4 to 45	4 to 38	4 to 28	7 to 11
Mode	6	8	6	5	6	N/A
Median (Q1, Q3)	7 (6, 8)	7 (6, 9)	6 (6, 7)	7 (5, 8)	6 (6, 8)	N/A

Median (Q1, Q3) shows the 50th, 25th, and 75th-centiles of the range of results. N/A: data not available for this time frame.

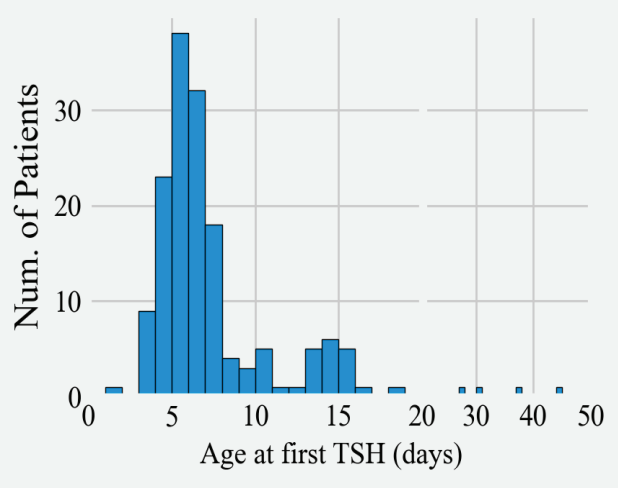

Figure 1. Age of infant in postnatal days at the time of first venous TSH measurement.

3.2. Timeliness of Monitoring

The effectiveness of longitudinal treatment was determined through serum TSH measurements for those with eligible lab tests within the pre-defined study time frame (Figure 2). The median (mode; range) number of TSH measurements during these times periods was 2 (2; 0 to 5) from 0 to 1 month of age, 4 (5; 0 to 12) from 1 to 6 months of age, 2 (2; 0 to 10) from 6 to 12 months of age, and 7 (5; 0 to 21) from 12 to 36 months of age.

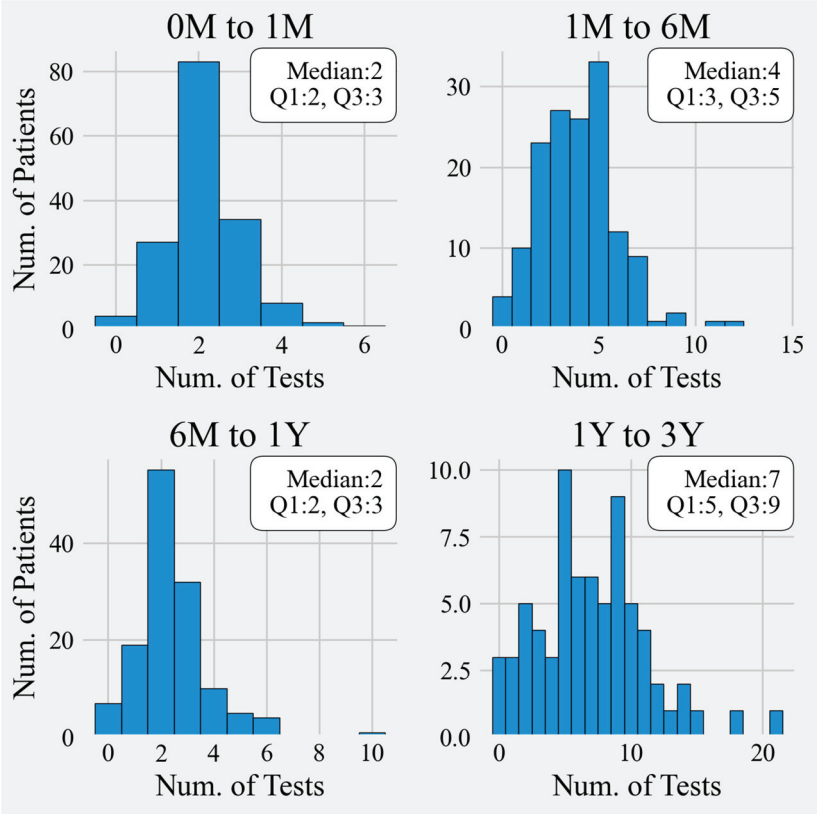

Figure 2. Number of TSH tests completed during specific age ranges. Abbreviations: TSH, Thyroid Stimulating Hormone; M, months; Y, years. Median (Q1, Q3) shows the 50th, 25th, and 75th-centiles of the range of results.

Within each year, there was at least one infant with infrequent laboratory monitoring. In 2014, one infant with an initial venous TSH of >150 mU/L had no subsequent labs between days 8 and 63 of age. In 2015, an infant with an initial TSH of 523 mU/L had no additional labs between days 6 and 315. In 2016, an infant with an initial TSH of 984 mU/L at 6 days of age had no subsequent labs. In 2017, there was an infant who did not have any labs past 17 days of age, and the TSH was 88 mU/L at that time. In 2018, one infant had no labs beyond 43 days of age despite being hypothyroid (TSH was 18 mU/L).

3.3. Appropriateness of Treatment

The frequencies of euthyroidism by the end of the first month of age are depicted in Figures 3 and 4. Overall, 42% had biochemical euthyroidism, but up to 51% of infants still manifested biochemical hypothyroidism at one month of age. Almost all of these were cases of subclinical hypothyroidism. The highest TSH levels at one month of age were observed among infants with the highest initial pretreatment TSH levels. Several among them still had TSH levels of ≥100 mU/L at one month of age. Five percent of the cohort was biochemically hyperthyroid at the end of the first month of age.

Once euthyroidism had been achieved, up to 67% of infants experienced some period of hypothyroidism (Table 2). Almost all of them had mild TSH elevations (with normal free T4 levels); they were followed up with timely monitoring and subsequently demonstrated to be within normal range. However, there were exceptions among this cohort who experienced protracted periods of severe hypothyroidism:

- In 2014, there was one infant who experienced prolonged hypothyroidism. This infant's initial venous TSH was >500. This infant's TSH level did not fall below 10 mU/L until day 437. Thereafter, this infant became euthyroid, which persisted until the last record at 1041 days.

- One infant born in 2015 did not become euthyroid until 89 days of age. Afterwards, this infant had fluctuating levels of hypothyroidism and euthyroidism on day 120 (TSH 24), became euthyroid on days 616 (TSH 126) to 734 (TSH 129), and then was euthyroid on days 778 (TSH 24) to 810 (TSH 23 mU/L).
- In 2016, an infant with a free T4 of 6.9 pmol/L was started on levothyroxine for CH. At 360 days of age, the TSH level was >150; and at 505 days of age, the TSH was 91 mU/L. There were no other thyroid function indices in between.

After becoming euthyroid, hyperthyroidism was frequently encountered. All of these were cases of subclinical hyperthyroidism.

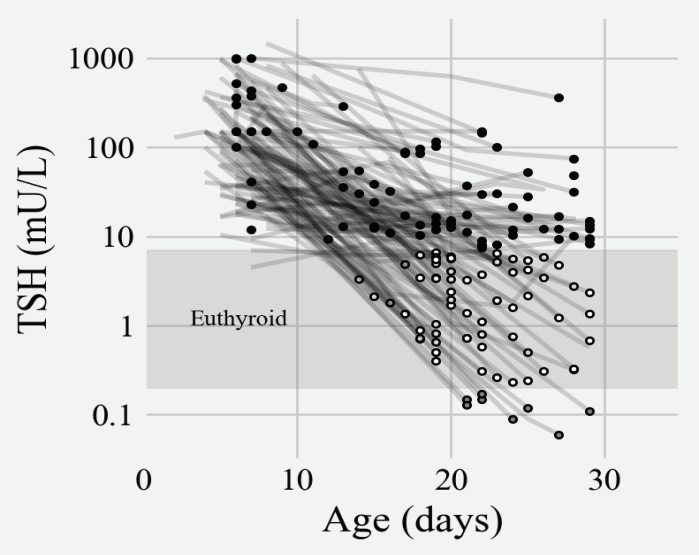

Figure 3. Range of initial and 1-month TSH levels. (Filled circles = hypothyroid; open circles = euthyroid; gray circles = hyperthyroid).

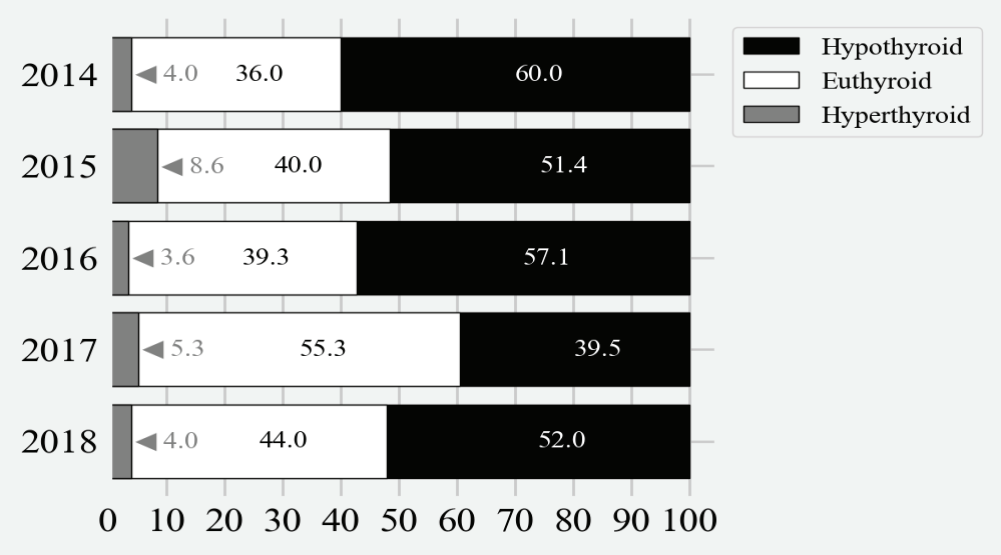

Figure 4. Frequencies (%) of euthyroidism, hypothyroidism, and hyperthyroidism at 1 month of age. Euthyroidism was defined as a TSH level between 0.2 and 7 mU/L, hypothyroidism defined as TSH > 7 mU/L, and hyperthyroidism defined as TSH < 0.2 mU/L. Year 2019 (infants born between 1 January and 31 March 2019) was not included in this analysis because there were insufficient data.

Table 2. Hypothyroidism (TSH > 7.0 mU/L) and hyperthyroidism (TSH < 0.2 mU/L) beyond 1 month of age after becoming euthyroid.

Year	2014 *n* = 27	2015 *n* = 35	2016 *n* = 30	2017 *n* = 39	2018 *n* = 23
Hypothyroidism after euthyroidism (*n*; %)	17 (63%)	21 (60%)	20 (67%)	24 (62%)	11 (48%)
TSH range (mU/L)	8.4 to 59.1	7.1 to 129.4	7.6 to >150	8.2 to 191.4	7.7 to 78.2
Hyperthyroidism after euthyroidism (*n*; %)	13 (45%)	22 (56%)	13 (37%)	16 (36%)	6 (21%)

Year 2019 (infants born between 1 January and 31 March 2019) was not included in this analysis because there were insufficient data.

4. Discussion

The most recent "Technical Report on the Screening and Management of Congenital Hypothyroidism" from the American Academy of Pediatrics (AAP) stresses the importance of clinical and laboratory follow-up of children with CH [19]. In addition to NBS, the management of CH requires timely confirmation of diagnosis and initiation of treatment and consistent follow-up. The major factors that determine long-term cognitive outcomes include the severity of CH, starting dose and timing of initiation of treatment with levothyroxine, and time to restoration of euthyroidism [20]. The goal is to initiate levothyroxine therapy as early as possible, generally by 2 weeks of age [19]. TSH levels should be kept within the age-specific reference range. There is no head-to-head comparison of one monitoring protocol over another, but normal cognitive outcomes have been achieved with monthly, bimonthly, and 3-monthly measurements in the first 2 to 3 years of life. In addition, it is recommended that more frequent monitoring happen during more rapid periods of growth, for example, in the first year of life [14].

We noticed an increased incidence of CH in Alberta over the last 20 years. Between 2002 and 2005, the incidence of CH in Alberta was reported to be approximately 1 in 3500; however, our study shows an incidence of 1:1470 [5]. An increased global incidence of CH over the past 2–3 decades has been reported also in the province of Quebec in Canada, as well as in France, New Zealand, Italy, Argentina, Greece, and the United States [21,22]. It is not completely clear what is causing this increase; most of these increases have been ascribed to the lowering of TSH cutoffs, therefore allowing for the detection of milder cases of CH. In some studies, changes in the ethnic composition of the screened population were also considered to be the cause of the increase [21,22].

Our study demonstrated that nearly all 160 term infants with CH detected through NBS had confirmation of their diagnosis within 16 days of age. There was an overall decrease in TSH levels between the time of the initial confirmatory serum sample and one month of age. Though we cannot with certainty state that this was due to levothyroxine therapy, the trend implies that treatment had been started. TSH measurements were most commonly measured 2 times between 0 and 1 month of age, 4 times between 1 and 6 months, 2 times between 6 and 12 months, and 7 times from 12 up to 36 months of age. The median counts within each time interval show that TSH monitoring generally happened as recommended by international guidelines.

However, our study also showed that the frequency of laboratory monitoring, as well as the attainment and sustainment of euthyroidism, varied widely. Some infants had a paucity of laboratory monitoring completed, while others experienced venipuncture frequently. We discovered several infants each year who were over 1 month of age before a TSH level was measured, had no laboratory testing beyond the first measurement, or went a year or more without any monitoring. Using retrospective administrative data limits us from accessing the clinical context within which care was happening. For instance, we are unable to ascertain whether infants without subsequent monitoring had moved out of

province. Nevertheless, further investigations are required to understand the circumstances of these cases.

Among those who had FT4 data available, almost all of their levels were within normal range by one month of age; however, the TSH levels of nearly half of the cohort exceeded 7 mU/L. In other words, subclinical hypothyroidism was common at 1 month of age and frequently recurred among those who eventually became euthyroid. TSH values above 5 mU/L are considered to be abnormal in infants over 3 months of age [19]. Because the hypothalamic-pituitary-thyroidal axis adapts finely to changes in serum free thyroid hormone levels, an increase in TSH suggests insufficiency of thyroid hormone. The 2020–2021 European guidelines recommend treatment with levothyroxine if the serum TSH concentration is 6 to 20 mU/L (with normal free T4) beyond the age of 21 days [14]. Whether mild subclinical hypothyroidism affects neurocognitive outcomes remains a question requiring further investigation. Gross and Van Vliet found that infants with recurrent episodes of subclinical hypothyroidism after 6 months of age had cognitive scores lower than their peers, a higher incidence of behavioral issues, and poorer school performance [2].

Higher initial doses of levothyroxine increase FT4 levels more rapidly but can lead to transient periods of hyperthyroidism. A retrospective study of a Polish CH newborn screening program between 2017 and 2021 demonstrated that 51 (58%) out of 88 patients were optimally treated, while approximately 25% were overtreated. The authors of this study suggested that overtreatment may have occurred among those with transient or milder forms of hypothyroidism who did not require as much exogenous thyroid hormone [23]. A belief exists among some clinicians that overtreatment may be more acceptable than undertreatment, but overtreatment may be associated with inattention, hyperactivity, anxiety, and deficits in cognitive function [24]. Long-term behavioral problems have been described in some cases, but the data are inconsistent with regards to the types and severity of behavioral problems.

The retrospective nature of our study, using data from administrative databases, precludes us from explaining the causes of infrequent TSH monitoring for some infants or why nearly half of the cohort experienced subclinical hypothyroidism. It can be inferred that the overall lowering of TSH levels after confirmation of diagnosis reflects that therapy was started; however, the timing of when levothyroxine therapy was prescribed and started could not be accurately ascertained from the administrative databases and is a limitation of this study. While inadequate therapy could be one reason for suboptimal outcomes, the reasons are likely to be multifactorial. These include the etiology of congenital hypothyroidism, with thyroid agenesis, ectopia, or hypoplasia likely to result in more severe disease; limited access to healthcare providers, laboratory facilities, and treatments; parental non-adherence due to a misunderstanding about the importance of treatment; and providers' lack of knowledge in management goals or confidence in interpreting lab results. Based on health insurance claims databases in the United States, Kemper and colleagues identified that approximately 40% of children with CH appeared to have discontinued treatment by 36 months after initiation of treatment [25]. Up to half of patients with a diagnosis of CH may be lost to follow-up [25–27]. The Region 4 Midwest Genetics Collaborative established the Congenital Hypothyroidism 3-Year Follow-Up Workgroup in 2011. From the Clinician Survey, clinicians reported that some patients had mild or transient hypothyroidism and had normal thyroid testing off medication; however, some parents had stopped treatment on their own. From the Parent Survey, only two-thirds of parents replied that they were satisfied with the level of medical education they received from their infants' provider [27].

Pediatric endocrinologists are involved in the initial assessment, but the long-term management of children with CH, which necessitates frequent follow-up and monitoring, may be conducted by pediatricians, nurse practitioners, or family physicians [28]. In a cross-sectional survey of primary care providers (PCPs) in California and Hawaii, the two most commonly perceived barriers to providing long-term care for patients with CH

were (1) that they need guidance or support from endocrinologists and (2) that they were not familiar with the CH treatment guidelines. The proportion of PCPs who correctly identified the recommended frequency of blood tests for three different age groups was no higher than 73% [26]. In Alberta, infants who screen positive for CH receive an initial consultation with a pediatric endocrinologist for confirmation of the diagnosis and initiation of treatment. Pediatric endocrinologists are based in the two largest cities—Edmonton and Calgary. Subsequent follow-up may be performed by a pediatrician or family doctor. The administrative codes in the databases were not sufficiently granular to distinguish the specialty of the ordering provider.

Alberta has been screening infants for CH for almost 50 years, and it is fair to conclude from our study that most infants are benefiting from timely and appropriate long-term management. However, the handful of infants with scant monitoring or prolonged hypothyroidism spotlights gaps in care that call out for changes to how long-term follow-up happens. Future studies can follow patients prospectively through their clinical journeys. The ANSP can develop a program to track longitudinally the clinical outcomes, particularly of those with the most severe disease. The ANSP has also developed a Clinical Algorithm tool that can be more consistently distributed to providers and parents at the time of diagnosis [16]. Solutions will require a multi-pronged approach and include eliminating physical and figurative barriers (e.g., geographic distance and administrative red tape) that prevent infants from accessing healthcare providers and laboratory facilities; systematic, initial education and periodic re-education with primary care providers and caregivers; and facilitating access to long-term outcomes data for the purposes of quality improvement.

5. Conclusions

It is imperative that the newborn screening process for CH evaluate the outcomes of infants who have confirmed CH as part of continuous quality improvement. More work needs to be performed to understand why some are experiencing prolonged periods of hypothyroidism or infrequent lab monitoring. There should be a process for identifying and supporting the management of infants whose thyroid hormone levels are significantly abnormal.

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References

1. Van Vliet, G.; Deladoey, J. Diagnosis, treatment and outcome of congenital hypothyroidism. *Endocr. Dev.* **2014**, *26*, 50–59.
2. Grosse, S.D.; van Vliet, G. Prevention of intellectual disability through screening for congenital hypothyroidism: How much and at what level? *Arch. Dis. Child.* **2011**, *96*, 374–379. [CrossRef]
3. Dussault, J.H. The anecdotal history of screening for congenital hypothyroidism. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 4332–4334. [CrossRef] [PubMed]
4. De Souza, A.; Wolan, V.; Battochio, A.; Christian, S.; Hume, S.; Johner, G.; Lilley, M.; Ridsdale, R.; Schnabl, K.; Tran, C.; et al. Newborn Screening: Current Status in Alberta, Canada. *Int. J. Neonatal Screen.* **2019**, *5*, 37. [CrossRef]

5. Alberta Health and Wellness. *Newborn Metabolic Screening in Alberta 2002–2005*; Alberta Health and Wellness, Public Health Division: Edmonton, AB, Canada, 2006.
6. American Academy of Pediatrics; Rose, S.R.; Section on Endocrinology and Committee on Genetics, American Thyroid Association; Brown, R.S.; Public Health Committee, Lawson Wilkins Pediatric Endocrine Society; Foley, T.; Kaplowitz, B.P.; Kaye, C.I.; Sundararajan, S.; Varma, S.K. Update of newborn screening and therapy for congenital hypothyroidism. *Pediatrics* **2006**, *117*, 2290–2303. [CrossRef] [PubMed]
7. US Preventive Services Task Force. Screening for congenital hypothyroidism: US Preventive Services Task Force reaffirmation recommendation. *Ann. Fam. Med.* **2008**, *6*, 166.
8. Toublanc, J.E. Guidelines for neonatal screening programs for congenital hypothyroidism. Working Group for Neonatal Screening in Paediatric Endocrinology of the European Society for Paediatric Endocrinology. *Acta Paediatr. Suppl.* **1999**, *88*, 13–14. [CrossRef]
9. Leger, J.; Olivieri, A.; Donaldson, M.; Torresani, T.; Krude, H.; van Vliet, G.; Polak, M.; Butler, G.; on behalf of ESPE-PES-SLEP-JSPE-APEG-APPES-ISPAE, and the Congenital Hypothyroidism Consensus Conference Group. European Society for Paediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. *Horm. Res. Paediatr.* **2014**, *81*, 80–103. [CrossRef] [PubMed]
10. Diaz, A.; Lipman Diaz, E.G. Hypothyroidism. *Pediatr. Rev.* **2014**, *35*, 336–347, quiz 348–349. [CrossRef]
11. Bongers-Schokking, J.J.; Resing, W.C.; de Rijke, Y.B.; de Ridder, M.A.; de Muinck Keizer-Schrama, S.M. Cognitive development in congenital hypothyroidism: Is overtreatment a greater threat than undertreatment? *J. Clin. Endocrinol. Metab.* **2013**, *98*, 4499–4506. [CrossRef]
12. Daneman, D.; Howard, N.J. Neonatal thyrotoxicosis: Intellectual impairment and craniosynostosis in later years. *J. Pediatr.* **1980**, *97*, 257–259. [CrossRef] [PubMed]
13. Dawrant, J.M.; Pacaud, D.; Wade, A.; Archer, S.; Bamforth, F.J. Informatics of newborn screening for congenital hypothyroidism in Alberta 2005–2008, flow of information from birth to treatment. *Can. J. Public Health* **2011**, *102*, 64–67. [CrossRef] [PubMed]
14. Van Trotsenburg, P.; Stoupa, A.; Leger, J.; Rohrer, T.; Peters, C.; Fugazzola, L.; Cassio, A.; Heinrichs, C.; Beauloye, V.; Pohlenz, J.; et al. Congenital Hypothyroidism: A 2020–2021 Consensus Guidelines Update—An ENDO-European Reference Network Initiative Endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. *Thyroid* **2021**, *31*, 387–419. [CrossRef] [PubMed]
15. Jonklaas, J.; Bianco, A.C.; Bauer, A.J.; Burman, K.D.; Cappola, A.R.; Celi, F.S.; Cooper, D.S.; Kim, B.W.; Peeters, R.P.; Rosenthal, M.S.; et al. Guidelines for the treatment of hypothyroidism: Prepared by the American thyroid association task force on thyroid hormone replacement. *Thyroid* **2014**, *24*, 1670–1751. [CrossRef] [PubMed]
16. Alberta Health Services, ANSP, CH Algorithm V2.1. Clinical Algorithm for Congenital Hypothyroidism Abnormal Screen Result © March 2023. Available online: https://www.albertahealthservices.ca/assets/info/hp/nms/if-hp-nms-ch-algorithm.pdf (accessed on 1 October 2023).
17. Available online: https://www.albertahealthservices.ca/assets/wf/lab/wf-lab-bulletin-thyroid-hormone-testing-changes.pdf (accessed on 1 October 2023).
18. Fisher, D.A.; Klein, A.H. Thyroid development and disorders of thyroid function in the newborn. *N. Engl. J. Med.* **1981**, *304*, 702–712. [CrossRef] [PubMed]
19. Rose, S.R.; Wassner, A.J.; Wintergerst, K.A.; Yayah-Jones, N.H.; Hopkin, R.J.; Chuang, J.; Smith, J.R.; Abell, K.; LaFranchi, S.H.; Section on Endocrinology Executive Committee; et al. Congenital Hypothyroidism: Screening and Management. *Pediatrics* **2023**, *151*, e2022060419. [CrossRef] [PubMed]
20. Balhara, B.; Misra, M.; Levitsky, L.L. Clinical monitoring guidelines for congenital hypothyroidism: Laboratory outcome data in the first year of life. *J. Pediatr.* **2011**, *158*, 532–537. [CrossRef]
21. Barry, Y.; Bonaldi, C.; Goulet, V.; Coutant, R.; Leger, J.; Paty, A.C.; Delmas, D.; Cheillan, D.; Roussey, M. Increased incidence of congenital hypothyroidism in France from 1982 to 2012, a nationwide multicenter analysis. *Ann. Epidemiol.* **2016**, *26*, 100–105.e4. [CrossRef] [PubMed]
22. Deladoey, J.; Ruel, J.; Giguere, Y.; Van Vliet, G. Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population-based study in Quebec. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 2422–2429. [CrossRef]
23. Lipska, E.; Lecka-Ambroziak, A.; Witkowski, D.; Szamotulska, K.; Mierzejewska, E.; Oltarzewski, M. Primary Congenital Hypothyroidism in Children Below 3 Years Old–Etiology and Treatment with Overtreatment and Undertreatment Risks, a 5-Year Single Centre Experience. *Front. Endocrinol.* **2022**, *13*, 895507. [CrossRef]
24. Bongers-Schokking, J.J.; Resing, W.C.M.; Oostdijk, W.; de Rijke, Y.B.; de Muinck Keizer-Schrama, S. Relation between Early Over- and Undertreatment and Behavioural Problems in Preadolescent Children with Congenital Hypothyroidism. *Horm. Res. Paediatr.* **2018**, *90*, 247–256. [CrossRef] [PubMed]
25. Kemper, A.R.; Ouyang, L.; Grosse, S.D. Discontinuation of thyroid hormone treatment among children in the United States with congenital hypothyroidism: Findings from health insurance claims data. *BMC Pediatr.* **2010**, *10*, 9. [CrossRef] [PubMed]
26. Sontag, M.K.; Sarkar, D.; Comeau, A.M.; Hassell, K.; Botto, L.D.; Parad, R.; Rose, S.R.; Wintergerst, K.A.; Smith-Whitley, K.; Singh, S.; et al. Case Definitions for Conditions Identified by Newborn Screening Public Health Surveillance. *Int. J. Neonatal Screen.* **2018**, *4*, 16. [CrossRef] [PubMed]

27. Wintergerst, K.A.; Eugster, E.; Andruszewski, K.; Kleyn, M.; Vanderburg, N.; Sockalosky, J.; Menon, R.; Linard, S.; Kingery, S.; Rose, S.R.; et al. Congenital Hypothyroidism 3-Year Follow-Up Project: Region 4 Midwest Genetics Collaborative Results. *Int. J. Neonatal Screen.* **2018**, *4*, 18. [CrossRef]
28. Rosenthal, N.A.; Bezar, E.; Mann, S.; Bachrach, L.K.; Banerjee, S.; Geffner, M.E.; Gottschalk, M.; Shapira, S.K.; Hasegawa, L.; Feuchtbaum, L. Primary Care Provider Management of Congenital Hypothyroidism Identified Through Newborn Screening. *Ann. Thyroid Res.* **2017**, *3*, 95–101.

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Impact of Lowering TSH Cut-Off on Neonatal Screening for Congenital Hypothyroidism in Minas Gerais, Brazil

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Abstract: A higher incidence of primary congenital hypothyroidism (CH) has been related to increased sensitivity in neonatal screening tests. The benefit of treatment in mild cases remains a topic of debate. We evaluated the impact of reducing the blood-spot TSH cut-off (b-TSH) from 10 (Group 2) to 6 mIU/L (Group 1) in a public neonatal screening program. During the study period, 40% of 123 newborns with CH (n = 162,729; incidence = 1:1323) had b-TSH between 6 and 10 mIU/L. Group 1 patients had fewer clinical signs (p = 0.02), lower serum TSH ($p < 0.01$), and higher free T4 ($p < 0.01$) compared to those in Group 2 at diagnosis. Reducing the b-TSH cut-off from 10 to 6 mIU/L increased screening sensitivity, allowing a third of diagnoses, mainly mild cases, not being missed. However, when evaluating the performances of b-TSH cut-offs (6, 7, 8, 9, and 10 mIU/L), the lower values were associated with low positive predictive values (PPVs) and unacceptable increased recall rates (0.57%) for a public health care program. A proposed strategy is to adopt a higher b-TSH cut-off in the first sample and a lower one in the subsequent samples from the same child, which yields a greater number of diagnoses with an acceptable PPV.

Keywords: congenital hypothyroidism; neonatal screening; cut-off; sensitivity

1. Introduction

Neonatal screening for congenital hypothyroidism (CH) is a milestone in the prevention of severe neurological sequelae, enabling the timely identification and treatment of affected newborns [1–3]. Immediately after screening was implemented, the incidence of the disease increased from 1:6700 to approximately 1:3500 live births. In recent decades, however, higher incidences have been reported, between 1:1400 and 1:3000, depending on the region, ethnicity, and screening methods [1,2,4–7].

The reason for this increase is still debated. Longer survival of premature and low-birthweight newborns, advanced maternal age, and ethnic changes in the population have been suggested as contributing factors [7–10]. Nonetheless, a decreased thyroid-stimulating hormone (TSH) cut-off at blood-spot screening (b-TSH) is considered the main factor for the increased incidence [2,11–13]. This rise occurs primarily, but not exclusively, because of the detection of mild cases with eutopic glands [4].

Treatment with levothyroxine for moderate to severe CH has unquestionable benefits. However, the natural course of the disease and the need for treatment in mild cases are

not fully understood [2,14–17]. This is one of the reasons why it is necessary to discuss the need and impact of decreasing the b-TSH cut-off level in neonatal screening programs for CH.

With this in mind, we evaluated the impact of b-TSH cut-off reduction on a government-funded neonatal screening program for CH.

2. Materials and Methods

This universal prospective cohort study was conducted between November 2021 and August 2022 in the Neonatal Screening Program funded by the Brazilian State of Minas Gerais (PTN-MG, acronym in Portuguese).

Minas Gerais is the 4th largest (588,383 km^2) and 2nd most populated Brazilian state, with 853 municipalities. It is located in the south-eastern region of Brazil. Approximately 18,000–20,000 newborns are screened per month by the program.

The study was approved by the Research Ethics Committee of Universidade Federal de Minas Gerais (CAAE 50311321.1.0000.5149), the parents or legal guardians received relevant information and signed an informed consent form.

Before the study, the PTN-MG used the protocol established by the Brazilian Ministry of Health to measure filter-paper bloodspot TSH (b-TSH) with a cut-off of 10 mIU/L. During the study, the protocol for newborn screening was modified, and the cut-off value for b-TSH was reduced from 10 mIU/L to 6 mIU/L.

The technical operation of the PTN-MG is carried out by the Núcleo de Ações e Pesquisa em Apoio Diagnóstico (NUPAD) [Center for Diagnostic Support Action and Research] of the Medical School of the Universidade Federal de Minas Gerais (FM-UFMG). General management and coordination are performed by the Health Secretariat of the State of Minas Gerais.

Bloodspots on filter paper (S&S 903®) are collected, between the third and fifth day of life, at primary healthcare units (90%) or birth hospitals (10%), from newborns who were not discharged by the fifth day due to relevant clinical conditions. There are 3744 collection points. Samples are sent via the postal service to the referenced laboratory at Nupad, and results are typically released within 24 h of sample receipt. Given the vast size of Minas Gerais, the average transportation time from sampling to arrival at the laboratory may vary, up to 5 days.

b-TSH is evaluated using the fluoroimmunoassay method (GSP Neonatal hTSH, Turku, Finland) at the NUPAD laboratory. According to the protocol, children with b-TSH $\geq$ 20 mIU/L were contacted by phone for an emergency appointment at the reference center to confirm the diagnosis. Those who presented borderline results (between 6 and 20 mIU/L) had a second sample immediately collected on a filter paper and were referred to an appointment if the result remained $\geq$6 mIU/L (Figure 1).

Extreme preterm babies (children born at fewer than 32 weeks of gestation or weighing less than 1500 g), newborns with hemodynamic instability, and those who received a transfusion before sample collection for screening, who were at risk for a false-negative result, underwent serial collections even if the first sample was normal (special protocols). In such instances, bloodspots are repeated at 10 and 30 days of life or 10 days after a blood transfusion. Newborns are closely monitored, with guidance provided to the local medical team by the pediatric endocrinologists of the program through phone contact with NUPAD.

The first consultations are conducted by the PTN-MG team at the reference center (Hospital das Clínicas—UFMG, in Belo Horizonte). Subsequent follow-up, including quarterly assessments up to 3 years of age, is carried out collaboratively at both the reference center and in the patient's municipality of residence.

Diagnosis is confirmed based on serum TSH (s-TSH—reference values (RV): 0.69 to 8.55 μIU/mL) and free thyroxine levels (fT4—RV: 0.89 to 1.76 ng/dL) by means of chemiluminescence immunometric assay (ICMA). Newborns with b-TSH between 6 and <10 mIU/L underwent thyroid ultrasound by one single trained radiologist, with a reference value for thyroid volume of 0.45 to 1.34 cm^3 [18].

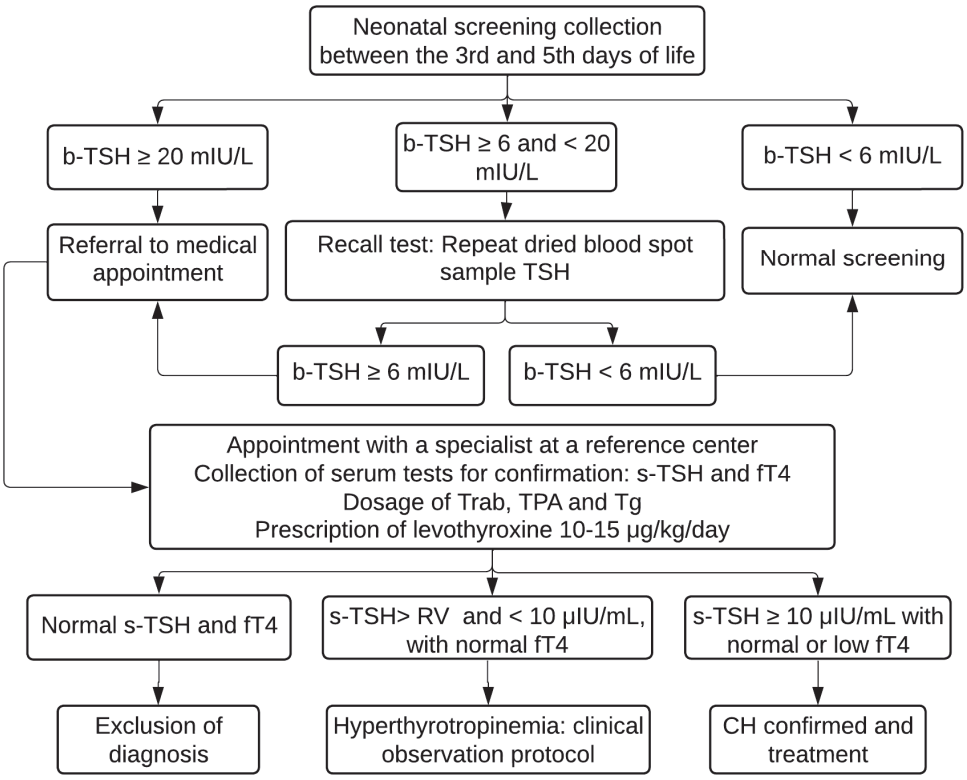

Figure 1. PTN-MG flowchart for diagnosis of congenital hypothyroidism between November 2021 and August 2022. b-TSH: filter-paper blood-spot TSH; s-TSH: serum TSH; fT4: free T4; Trab: TSH receptor autoantibodies; TPA: Thyroid Peroxidase Antibody; Tg: Thyroglobulin; RV: reference value.

The criteria used to confirm cases and initiate treatment were s-TSH $\geq$ 10 µIU/mL and normal or low fT4 or borderline s-TSH (above the reference value, but lower than 10 µIU/mL) associated with low fT4. Patients diagnosed based on this last criterion were assessed for central CH.

Patients presenting with s-TSH $\geq$ 10 µIU/mL and fT4 below the reference value were classified as decompensated CH.

According to the sample size calculation, with 95% confidence, a minimum sample size was established for 60 detected cases of CH and at least 150,000 negative tests.

The analyses were performed using software R 4.1.2 and IBM SPSS Statistics 26. A significance level of 5% was considered for all the tests. Variables were verified for normality using the Kolmogorov–Smirnov or Shapiro–Wilk tests. Continuous variables were characterized by medians, minimum and maximum values, and percentiles. Comparisons between continuous variables were performed using the Kruskal–Wallis test with the Bonferroni post-hoc test. Categorical variables were represented by their absolute values and percentages and were compared using Pearson's chi-square test. The incidence of CH was calculated, as well as the recall rates for new sample collections and calls for appointments in the period. The sensitivity and specificity of b-TSH cut-off values of 6, 7, 8, 9, and 10 mIU/L were used to create the ROC curve. Moreover, the respective positive and negative predictive values (PPVs and NPVs) were calculated to compare the performance. Patients who were screened using special protocols were excluded.

For clinical and laboratory comparisons, subjects referred for medical appointments during the study period were divided into two groups, stratified according to b-TSH values at first sampling: (1) newborns with b-TSH between 6 and <10 mIU/L and (2) newborns with b-TSH $\geq$ 10 mIU/L.

3. Results

During the study period, 162,729 newborns were screened in the PTN-MG, and 3070 by using special protocols. A total of 123 cases of CH were diagnosed and treated with levothyroxine, and 50 of them were identified due to a change in the b-TSH cut-off (Figure 2). The incidence for the cut-off of 6 mIU/L was 1 case for every 1323 newborns, whereas for the cut-off of 10 mIU/L, it was 1:2229 in the study period.

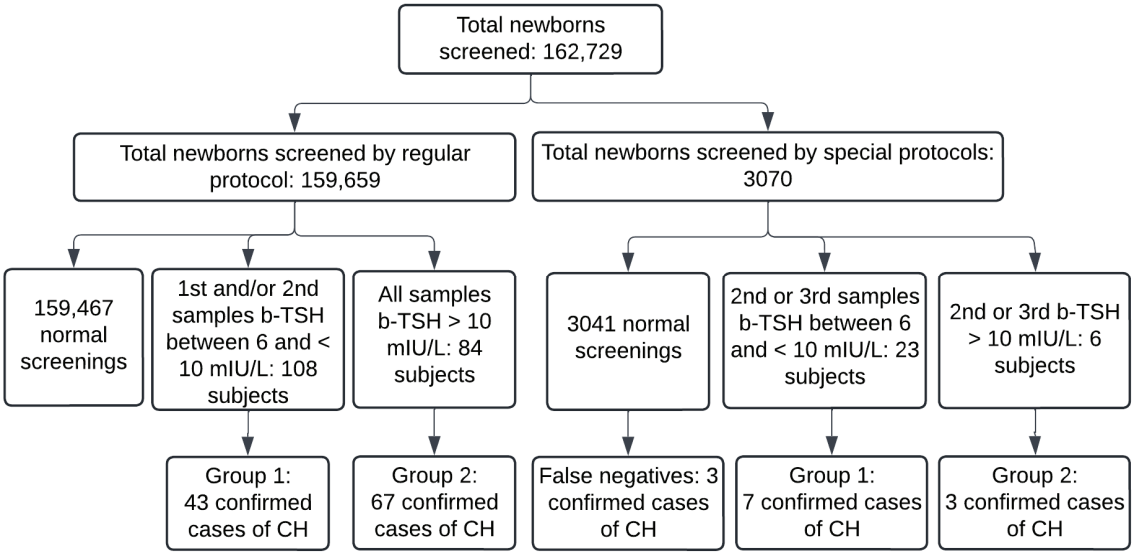

Figure 2. Flowchart of cases of congenital hypothyroidism diagnosed between November 2021 and August 2022 in Minas Gerais. b-TSH: filter-paper blood-spot TSH; CH: congenital hypothyroidism. Normal screenings include subjects who had a normal first sample and those whose screening was normalized in the second sample. False negatives: three confirmed cases diagnosed by clinical signs in the period.

The recall rate was calculated for a b-TSH cutoff of 6 mIU/L. The recall rate for a second sample of neonatal screenings was 0.57%, and for medical appointments was 0.08%.

The area under the ROC curve for the first sample was 0.999, with a 95% confidence interval (95% CI) between 0.999 and 0.999; for the second sample, it was 0.991 (CI 0.986–0.996). The projected performances for the different cut-offs are summarized in Table 1. All the cut-offs assessed had adequate specificity and NPV. Lower cut-offs were related to better sensitivity, but with a decrease in PPV and increase in recall rates.

Group 1 consisted of 50 patients, with 27.6% preterm children (<37 weeks of gestation) and Group 2 had 58 patients, 20% premature. The prematurity rate did not differ significantly between the groups ($p = 0.378$). Newborns called for a medical appointment showed a median of b-TSH in the first neonatal screening sample of 7.44 mIU/L (Q1, 6.02; Q3, 9.91) in Group 1, and of 39.88 mIU/L (Q1, 10.90; Q3, 313) in Group 2 ($p < 0.01$). First samples were collected at a median age of 5 days for both groups. In the second sample, Group 1 had a b-TSH median of 9.53 mIU/L (Q1, 4.69; Q3, 24.34), at a median age of 14 days, and Group 2, at 13 days, had a median of 21.40 mIU/L (Q1, 10.68; Q3, 77.46) ($p < 0.01$). Patients with a b-TSH $\geq$ 20 mIU/L in the first sample were referred for emergency appointments.

In Group 1, 83% of patients showed at least one clinical sign of CH, such as prolonged jaundice, dry skin, poor weight gain, large fontanelles, and cranial sutures, and 94% in Group 2. Group 2 patients presented significantly more clinical signs than Group 1 ($p = 0.02$), even though they were assessed in a medical appointment earlier (median age of 16 vs. 25 days old).

Table 1. Projected performance for different b-TSH cut-offs for congenital hypothyroidism in neonatal screening between November 2021 and August 2022, in Minas Gerais State.

b-TSH Cut-Off (mIU/L) for the 1st Sample (*n* = 159,659)	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**Recall Rate for 2nd Sample**
6	100% (100–100)	99.50% (99.46–99.53)	11.87% (11.71–12.03)	100.00% (100–100)	0.57%
7	85.30% (85.15–85.49)	99.70% (99.67–99.73)	16.88% (16.69–17.06)	99.99% (99.99–99.99)	0.35%
8	78.90% (78.7–79.1)	99.80% (99.82–99.86)	24.93% (24.72–25.14)	99.99% (99.98–99.99)	0.22%
9	70.60% (70.42–70.87)	99.90% (99.89–99.92)	34.69% (34.45–34.92)	99.98% (99.97–99.99)	0.14%
10	64.20% (63.99–64.46)	99.90% (99.93–99.95)	41.42% (41.18–41.66)	99.98% (99.97–99.98)	0.11%
b-TSH cut-off (mIU/L) for the 2nd sample (*n* = 793)	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**Recall Rate for Appointment**
6	98.20% (97.33–99.16)	98.10% (97.15–99.05)	80.00% (77.22–82.78)	99.86% (99.60–100)	0.08%
7	80.70% (77.95–83.45)	98.10% (97.15–99.05)	76.67% (73.72–79.61)	98.50% (97.65–99.35)	0.07%
8	73.70% (68.80–75.06)	98.40% (97.49–99.25)	77.36% (74.45–80.27)	97.84% (96.83–98.85)	0.07%
9	64.90% (61.59–68.23)	98.90% (98.19–99.63)	82.22% (79.56–84.88)	97.33% (96.20–98.45)	0.06%
10	59.60% (56.23–63.06)	99.20% (98.56–99.81)	85.00% (82.51–87.49)	96.95% (95.75–98.14)	0.06%

b-TSH: filter-paper blood-spot TSH; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value.

Patients in Group 1 presented significantly lower levels of s-TSH and significantly higher levels of fT4 than those in Group 2 ($p < 0.001$ for both analyses), as summarized in Table 2. 21% of patients in Group 1 presented fT4 below the reference values used in the program.

Table 2. Serum tests of congenital hypothyroidism newborns between 2021 and 2022 in Minas Gerais State.

	Serum TSH		**Free T4**	
	Group 1 (b-TSH 6–9.9 mIU/L)	**Group 2 (b-TSH >= 10 mIU/L)**	**Group 1 (b-TSH 6–9.9 mIU/L)**	**Group 2 (b-TSH >= 10 mIU/L)**
Median	14.52 *	177.7 *	1.14 *	0.56 *
Minimum	10.08	10.4	0.40	0.10
Maximum	193.65	1370.0	2.54	1.68
25th percentile	11.72	47.81	0.93	0.27
75th percentile	27.62	394.55	1.37	0.99

b-TSH: filter-paper blood-spot TSH. Serum TSH: reference value = 0.69 to 8.55 µIU/mL; Free T4: reference value = 0.89 to 1.76 ng/dL. Group 1: $n = 50$; Group 2: $n = 58$. * $p < 0.001$—Kruskal–Wallis test.

A total of 18 patients from Group 1 underwent thyroid ultrasound upon diagnosis: three (16.7%) were suggestive of dysgenesis—two with hypoplasia, and one who showed

hemiagenesis—and 15 (83.3%) were inconclusive—two with goiter and 13 with normal thyroid. Patients in Group 2 did not undergo ultrasound at the time of diagnosis. All patients in both groups will be re-evaluated at the age of 3 years with imaging tests after temporarily discontinuing medication.

4. Discussion

The decrease in the b-TSH cut-off from 10 to 6 mIU/L represented a significant increase in the test sensitivity, allowing the detection of more newborns with CH, but, also, a high rate of false-positive cases. The unequivocal benefits of treating children preemptively and reports of undiagnosed CH with current cut-offs of b-TSH have led many newborn screening programs to reduce the thresholds, resulting in a higher incidence of the disease [2,11–13].

The incidence of CH in Brazil ranges from 1:2500 to 1:4800 of live births with a b-TSH cut-off of 10 mIU/L [19]. Similar to what was found by the present study, the incidence of CH rises sharply to 1:950–1:1560 [20–22] in some states that reduced the cut-offs to 4.5 to 6 mIU/L.

The same have been reported in several parts of the world, with identification of mild CH cases and reduction in percentage of dysgenetic glands [4,11].

In accordance with these reports, we observed a greater proportion of patients with less severe conditions, with few or no symptoms and compatible laboratory findings. Most patients assessed did not present anatomical abnormalities on thyroid ultrasound, a frequent finding related to CH diagnoses with thyroid gland in situ after a reduction in the cut-off points [4,11,16,23,24]. In a survey conducted at the PTN-MG between 2018 and 2019, with 44 children under the unchanged protocol, the proportion of newborns with a thyroid with no anatomical alterations on ultrasound in the first appointment was 58%, which is lower than the current findings.

However, more severe cases of CH have been reported with lower cut-off points, and approximately 40% of patients with permanent and decompensated CH upon diagnosis were found with b-TSH between 8 and 10 mIU/L [25].

We observed although most newborns in Group 1 presented mild laboratory alterations, 21% of the sample presented decompensated CH, which is an unequivocal indication for treatment in all consensus on the disease [1,3,26].

We show that if the cut-off of 10 mIU/L had been kept, 40% of newborns would not have been diagnosed with the disease, which seems an unacceptable rate. A significant number of children with transient or permanent CH being left undiagnosed using the cut-off of 10 mIU/L (18%) led the authors suggesting a value of 6 mIU/L to be more appropriate for the screening program in the United Kingdom [13].

On the other hand, a problem related to the reduction in the b-TSH cut-off is a great number of children called for medical assessment.

Keeping higher cut-offs, as seen in some high income countries, can reduce the number of false-positive results [27]. However, active vigilance and quality infant care must be guaranteed to diagnose possible cases missed during screening. A Swedish study showed that using a b-TSH cut-off of 15 mIU/L, 50% of detected CH cases had come back negative during neonatal screening and were identified during pediatric care [28]. Taking care of these children at the right time is certainly not possible in many regions.

There is no consensus on the ideal recall rate. Just as for the cut-off value, this rate depends on several factors, such as the geographical coverage of the program, available infrastructure, and financial resources, among others. The worldwide rate of calls for medical appointments varies between 0.01% and 13.3%, depending on the adopted b-TSH cut-off [29].

We observed the reduction in the b-TSH cut-off was associated with a 5.2-fold increase in the rate of recall for a second sample, and as the result was normal for most children, the rate of calls for appointments increased by 30%. This is an undesirable consequence. Even if the second sample is collected close to the child's residence and the local agents are

trained to inform families about the need for recollection, there may be a negative impact. Furthermore, potential extra cost may be imposed to the screening program, and should be evaluated in the future.

A similar reduction, from 10 to 6 mIU/L in the United Kingdom, led the recall rate to increase from 0.08% to 0.23% [12]. The recall rate increased in all reported programs that implemented a reduction in the cut-off screening [30,31], up to 10 times [24,32]. High recall rates represent higher costs for screening programs, in addition to a higher number of healthy children undergoing needless exams and appointments, with a relevant emotional impact for the patients and their families.

In the United States, costs incurred from repeated tests can reach up to USD 2 million annually [29]; the burden can be expected to be bigger for disadvantaged populations. It is clear, however, that the lower the recall rate—without leaving sick children undiagnosed—the better.

In addition to the costs, a definition of the appropriate cut-off point for a neonatal screening program must consider the methods used; the median age of the newborns during sample collection; ethnicity of the population and degree of iodine sufficiency; the capacity to process the tests; the availability of specialized centers; and the socioeconomic and cultural factors. This definition should aim for a sensitivity close to 100%, but without overburdening the program with too many false-positive results.

Herein, the different cut-off points presented good specificity and a high NPV, which were expected based on the low prevalence of CH in the population [29]. Therefore, sensitivity and PPV assessments are more important for suitability analysis. Despite a higher sensitivity of the 6 mIU/L cut-off in comparison to the 10 mIU/L, the low PPV in the first sample—which generated over 88% of false-positive results—is not attractive to a government-funded program. In Australia, reducing the cut-off from 15 to 8 mIU/L caused the PPV to drop from 74.3% to 12.8% [32]. The same was reported in Brazilian regions—Rio de Janeiro and Campinas—which showed false-positive results close to 90% [20,33].

We sought better results to enhance the rate of CH diagnosis without significantly burdening the program or penalizing families. Analyzing the performances of the different cut-offs, we found an intermediate value of 8 mIU/L was related to good sensitivity and a PPV over two times higher than the cut-off of 6 mIU/L, which potentially makes it more adequate for the first sample. Thus, we opted to adopt different thresholds for the first and subsequent samples; 8 mIU/L for the first and 6 mIU/L for subsequent ones.

Although a higher prevalence of preterm infants could have been expected in Group 1, the similar number of preterm newborns observed in both groups precludes any effect of prematurity on the performance analysis of different b-TSH cut-off points, potentially increasing false-negative results.

One strategy to enhance the effectiveness of screening programs is re-screening extremely preterm and very low birth weight infants to prevent overlooking delayed TSH rise [1], and to reduce the recall rate for false-positive children with borderline results, without delaying the assessment of urgent cases [34].

Another one is the use of lower cut-off points only for the second sample, when recommended [34,35]. Decreasing the cut-off only in the second sample doubled the incidence of newborns diagnosed with dyshormonogenesis and dysgenesis [16], and allowed the diagnosis of 43.7% of CH cases in another report [36]. The use of these lower cut-off points takes into consideration the physiological changes of thyroid function, increase in TSH in the first 48 h after birth, and progressive decrease during the first weeks of life. This strategy appeared to be the best choice for improving our program, as the cut-off point of 6 mIU/L in the first sample resulted in an excessive number of false positives, although its use in subsequent samples increased test sensitivity without compromising PPV.

The disadvantages of using different cut-off points in the subsequent samples include the additional complexity of the screening process, which requires different criteria to interpret results according to the sample. This can increase the possibility of interpretation

errors and demands more training of the healthcare professionals involved in the program. To address this issue, the PTN-MG has a supportive team that receives periodic training.

The main objective of CH screening is to prevent intellectual deficit associated with the disease. Therefore, the main argument of authors who are against reducing cut-off points is that the benefit of treatment for very mild and subclinical cases—which comprise the majority of cases that have been identified—is still under investigation. The results obtained in the treatment of moderate to severe CH since screenings were implemented should not be simply extrapolated [37,38]. Natural evolution studies have been conducted to show the developmental impacts suffered by children with untreated mild CH, but results are still conflicting. Some reports suggest differences in the neurodevelopment of children with borderline b-TSH were not clinically relevant to recommend changes in the cut-off [39–41], while others show that these children had an increased risk of poor school performance, developmental changes, reduced memory span, and a higher chance to develop special needs [42,43]. This assessment is complex since cognitive deficits can be associated to multiple factors, including socioeconomic and environmental factors [44,45].

On the other hand, excessive treatment in CH patients appears to be linked to unfavorable cognitive outcomes [46–48]. There is still no consensus on this matter; further research is needed.

The main limitation of this study is a potential underestimation of false-negative cases since all patients with a neonatal b-TSH < 6 mIU/L were considered normal. Furthermore, few patients underwent thyroid ultrasound. However, the sample size and rigorous protocol are strengths supporting the results found are highly consistent.

5. Conclusions

There was an increase in the diagnosis of CH after reducing the b-TSH cut-off to 6 mIU/L, mainly attributed to the detection of children with mild cases. Maintaining a cut-off of 10 mIU/L would have resulted in 40% of newborns with CH going undiagnosed, which appears to be an unacceptable rate. Since the b-TSH cut-off of 6 mIU/L for the first sample exhibited a very low PPV, an intermediate value of 8 mIU/L is proposed, along with a threshold of 6 mIU/L for subsequent samples, to achieve the expected results. Continuous longitudinal monitoring of newborn screening programs utilizing lower cut-off points is essential to evaluate their efficacy over time.

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Informed Consent Statement: Informed consent was obtained from all parents/legal guardians of the patients involved in the study. Written informed consent has been obtained from parents/legal guardians of the patients to publish this paper.

Data Availability Statement: Data are not available due to ethical reasons. Further enquiries can be directed to the corresponding author.

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References

1. Trotsenburg, P.V.; Stoupa, A.; Léger, J.; Rohrer, T.; Peters, C.; Fugazzola, L.; Cassio, A.; Heinrichs, C.; Beauloye, V.; Pohlenz, J.; et al. Congenital Hypothyroidism: A 2020–2021 Consensus Guidelines Update—An ENDO-European Reference Network (ERN) initiative endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. *Thyroid* **2021**, *31*, 387–419. [CrossRef] [PubMed]
2. Wassner, A.J.; Brown, R.S. Congenital hypothyroidism: Recent advances. *Curr. Opin. Endocrinol. Diabetes Obes.* **2015**, *22*, 407–412. [CrossRef] [PubMed]
3. Nagasaki, K.; Minamitani, K.; Anzo, M.; Adachi, M.; Ishii, T.; Onigata, K.; Kusuda, S.; Harada, S.; Horikawa, R.; Minagawa, M.; et al. Guidelines for Mass Screening of Congenital Hypothyroidism (2014 revision) Mass Screening Committee, Japanese Society for Pediatric Endocrinology, and Japanese Society for Mass Screening. *Clin. Pediatr. Endocrinol.* **2015**, *24*, 107–133. [PubMed]
4. Chiesa, A.; Prieto, L.; Mendez, V.; Papendieck, P.; Calcagno, M.D.L.; Gruñeiro-Papendieck, L. Prevalence and etiology of congenital hypothyroidism detected through an argentine neonatal screening program (1997–2010). *Horm. Res. Paediatr.* **2013**, *80*, 185–192. [CrossRef] [PubMed]
5. Barry, Y.; Bonaldi, C.; Goulet, V.; Coutant, R.; Léger, J.; Paty, A.C.; Delmas, D.; Cheillan, D.; Roussey, M. Increased incidence of congenital hypothyroidism in France from 1982 to 2012: A nationwide multicenter analysis. *Ann Epidemiol* **2016**, *26*, 100–105.e4. [CrossRef] [PubMed]
6. Peters, C.; Brooke, I.; Heales, S.; Ifederu, A.; Langham, S.; Hindmarsh, P.; Cole, T.J. Defining the newborn blood spot screening reference interval for TSH: Impact of ethnicity. *J. Clin. Endocrinol. Metab.* **2016**, *101*, 3445–3449. [CrossRef] [PubMed]
7. Gu, Y.H.; Kato, T.; Harada, S.; Inomata, H.; Aoki, K. Time Trend and Geographic Distribution of Treated Patients with Congenital Hypothyroidism Relative to the Number of Available Endocrinologists in Japan. *J. Pediatr.* **2010**, *157*, 153–157. [CrossRef] [PubMed]
8. Heather, N.L.; Derraik, J.G.B.; Webster, D.; Hofman, P.L. The impact of demographic factors on newborn TSH levels and congenital hypothyroidism screening. *Clin. Endocrinol.* **2019**, *91*, 456–463. [CrossRef] [PubMed]
9. Albert, B.B.; Cutfield, W.S.; Webster, D.; Carll, J.; Derraik, J.G.B.; Jefferies, C.; Gunn, A.J.; Hofman, P.L. Etiology of increasing incidence of congenital hypothyroidism in New Zealand from 1993–2010. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 3155–3160. [CrossRef]
10. Harris, K.B.; Pass, K.A. Increase in congenital hypothyroidism in New York State and in the United States. *Mol. Genet. Metab.* **2007**, *91*, 268–277. [CrossRef]
11. Olivieri, A.; Fazzini, C.; Medda, E. Multiple factors influencing the incidence of congenital hypothyroidism detected by neonatal screening. *Horm. Res. Paediatr.* **2015**, *83*, 86–93. [CrossRef] [PubMed]
12. Korada, S.M.; Pearce, M.; Ward, M.P.; Avis, E.; Turner, S.; Wastell, H.; Cheetham, T. Difficulties in selecting an appropriate neonatal thyroid stimulating hormone (TSH) screening threshold. *Arch. Dis. Child.* **2010**, *95*, 169–173. [CrossRef] [PubMed]
13. Langham, S.; Hindmarsh, P.; Krywawych, S.; Peters, C. Screening for Congenital Hypothyroidism: Comparison of Borderline Screening Cut-Off Points and the Effect on the Number of Children Treated with Levothyroxine. *Eur. Thyroid. J.* **2013**, *2*, 180–186. [CrossRef] [PubMed]
14. Cherella, C.E.; Wassner, A.J. Congenital hypothyroidism: Insights into pathogenesis and treatment. *Int. J. Pediatr. Endocrinol.* **2017**, *2017*, 11. [CrossRef] [PubMed]
15. Deladoëy, J.; Van Vliet, G. The changing epidemiology of congenital hypothyroidism: Fact or artifact? *Expert. Rev. Endocrinol. Metab.* **2014**, *9*, 387–395. [CrossRef] [PubMed]
16. Deladoëy, J.; Ruel, J.; Giguère, Y.; Van Vliet, G. Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population-based study in Québec. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 2422–2429. [CrossRef] [PubMed]
17. Connelly, K.J.; Lafranchi, S.H. Detection of neonates with mild congenital hypothyroidism (primary) or isolated hyperthyrotropinemia: An increasingly common management dilemma. *Expert. Rev. Endocrinol. Metab.* **2014**, *9*, 263–271. [CrossRef] [PubMed]
18. Freire, R.; Monte, O.; Tomimori, E.K.; Catarino, R.M.; Sterza, T.; Rocha, T.; Pereira, K.C.C.; Mattos, H.S.; Fagundes, L.B.; Liberato, M.M.; et al. Sonographic evaluation of the thyroid size in neonates. *J. Clin. Ultrasound* **2015**, *43*, 224–229. [CrossRef] [PubMed]
19. Ministério da Saúde. *Protocolo Clínico e Diretrizes Terapêuticas para Hipotireoidismo Congênito*; Portaria SAS/MS no. 1161, de 18 de Novembro de 2015; Ministério da Saúde: Brasilia, Brasil, 2015; pp. 1–8.
20. Barone, B.; Lopes, C.d.L.S.; Tyszler, L.S.; do Amaral, V.B.; Zarur, R.H.C.; Paiva, V.N.; Leite, D.B.; Meirelles, R.M. Avaliação do valor de corte de TSH em amostras de filtro na triagem neonatal para diagnóstico de hipotireoidismo congênito no programa "Primeiros Passos"—IEDE/RJ. *Arq. Bras. Endocrinol. Metabol.* **2013**, *57*, 57–61. [CrossRef]
21. Nascimento, M.L.; Nascimento, A.L.; Dornbusch, P.; Ohira, M.; Simoni, G.; Cechinel, E.; Linhares, R.M.M.; Lee, J.V.D.S.; Silva, P.C.A. Impact of the reduction in TSH cutoff level to 6 mIU/L in neonatal screening for congenital hypothyroidism in Santa Catarina: Final results. *Arch. Endocrinol. Metab.* **2020**, *64*, 816–823. [CrossRef]
22. Matos, D.M.; Ramalho, R.J.R.; Carvalho, B.M.; Almeida, M.A.C.T.; Passos, L.F.D.; Vasconcelos, T.T.S.; Melo, E.V.; Oliveira, C.R.P.; Santos, E.G.; Resende, K.F.; et al. Evolution to permanent or transient conditions in children with positive neonatal TSH screening tests in Sergipe, Brazil. *Arch. Endocrinol. Metab.* **2016**, *60*, 450–456. [CrossRef] [PubMed]

23. Corbetta, C.; Weber, G.; Cortinovis, F.; Calebiro, D.; Passoni, A.; Vigone, M.C.; Beck-Peccoz, P.; Chiumello, G.; Persani, L. A 7-year experience with low blood TSH cutoff levels for neonatal screening reveals an unsuspected frequency of congenital hypothyroidism (CH). *Clin. Endocrinol.* **2009**, *71*, 739–745. [CrossRef] [PubMed]
24. Mengreli, C.; Kanaka-Gantenbein, C.; Girginoudis, P.; Magiakou, M.A.; Christakopoulou, I.; Giannoulia-Karantana, A.; Chrousos, G.P.; Dacou-Voutetakis, C. Screening for congenital hypothyroidism: The significance of threshold limit in false-negative results. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 4283–4290. [CrossRef] [PubMed]
25. McGrath, N.; Hawkes, C.P.; Mayne, P.; Murphy, N.P. Permanent Decompensated Congenital Hypothyroidism in Newborns with Whole-Blood Thyroid-Stimulating Hormone Concentrations between 8 and 10 mU/L: The Case for Lowering the Threshold. *Horm. Res. Paediatr.* **2018**, *89*, 265–270. [CrossRef]
26. Rose, S.R.; Wassner, A.J.; Wintergerst, K.A.; Yayah-Jones, N.H.; Hopkin, R.J.; Chuang, J.; Smith, J.R.; Abell, K.; LaFranchi, S.H. American Academy of Pediatrics Section on Endocrinology, AAP Council on Genetics, Pediatric Endocrine Society, American Thyroid Association. Technical report. Congenital hypothyroidism: Screening and management. *Pediatrics* **2023**, *151*, e2022060420. [CrossRef] [PubMed]
27. Danner, E.; Niuro, L.; Huopio, H.; Niinikoski, H.; Viikari, L.; Kero, J.; Jääskeläinen, J. Incidence of primary congenital hypothyroidism over 24 years in Finland. *Pediatr. Res.* **2022**, *93*, 649–653. [CrossRef] [PubMed]
28. Gunnerbeck, A.; Lundholm, C.; von Döbeln, U.; Zetterström, R.H.; Almqvist, C.; Nordenström, A. Neonatal screening for congenital hypothyroidism in Sweden 1980–2013: Effects of lowering the thyroid-stimulating hormone threshold. *Eur. J. Endocrinol.* **2023**, *188*, 536–546. [CrossRef]
29. Mehran, L.; Khalili, D.; Yarahmadi, S.; Amouzegar, A.; Mojarrad, M.; Ajang, N.; Azizi, F. Worldwide recall rate in newborn screening programs for congenital hypothyroidism. *Int. J. Endocrinol. Metab.* **2017**, *15*, e55451. [CrossRef] [PubMed]
30. Anne, R.P.; Rahiman, E.A. Congenital hypothyroidism in India: A systematic review and meta-analysis of prevalence, screen positivity rates, and etiology. *Lancet Reg. Health Southeast Asia* **2022**, *5*, 100040. [CrossRef]
31. Simonetti, S.; D'Amato, G.; Esposito, B.; Chiarito, M.; Dentico, D.; Lorè, T.; Cardinali, R.; Russo, S.; Laforgia, N.; Faienza, M.F. Congenital hypothyroidism after newborn screening program reorganization in the Apulia region. *Ital. J. Pediatr.* **2022**, *48*, 131. [CrossRef]
32. Yu, A.; Alder, N.; Lain, S.J.; Wiley, V.; Nassar, N.; Jack, M. Outcomes of lowered newborn screening thresholds for congenital hypothyroidism. *J. Paediatr. Child. Health* **2023**, *59*, 955–961. [CrossRef] [PubMed]
33. Christensen-Adad, F.C.; Mendes-dos-Santos, C.T.; Goto, M.M.F.; Sewaybricker, L.E.; D'Souza-Li, L.F.R.; Guerra-Junior, G.; Morcillo, A.M.; Lemos-Marini, S.H.V. Neonatal screening: 9% of children with filter paper thyroid-stimulating hormone levels between 5 and 10 μIU/mL have congenital hypothyroidism. *J. Pediatr.* **2017**, *93*, 649–654. [CrossRef] [PubMed]
34. Odenwald, B.; Brockow, I.; Hanauer, M.; Lüders, A.; Nennstiel, U. Is Our Newborn Screening Working Well? A Literature Review of Quality Requirements for Newborn Blood Spot Screening (NBS) Infrastructure and Procedures. *Int. J. Neonatal Screen.* **2023**, *9*, 35. [CrossRef] [PubMed]
35. Dorreh, F.; Chaijan, P.Y.; Javaheri, J.; Zeinalzadeh, A.H. Epidemiology of congenital hypothyroidism in Markazi Province, Iran. *J. Clin. Res. Pediatr. Endocrinol.* **2014**, *6*, 105–110. [CrossRef]
36. Caiulo, S.; Corbetta, C.; Di Frenna, M.; Medda, E.; De Angelis, S.; Rotondi, D.; Vincenzi, G.; de Filippis, T.; Patricelli, M.G.; Persani, L.; et al. Newborn screening for congenital hypothyroidism: The benefit of using differential TSH cutoffs in a two-screen program. *J. Clin. Endocrinol. Metab.* **2021**, *106*, e338–e349. [CrossRef]
37. Krude, H.; Blankenstein, O. Treating patients not numbers: The benefit and burden of lowering TSH newborn screening cut-offs. *Arch. Dis. Child.* **2011**, *96*, 121–122. [CrossRef] [PubMed]
38. Lain, S.; Trumpff, C.; Grosse, S.D.; Olivieri, A.; Van Vliet, G. Are lower TSH cutoffs in neonatal screening for congenital hypothyroidism warranted? *Eur. J. Endocrinol.* **2017**, *177*, D1–D12. [CrossRef]
39. Trumpff, C.; se Schepper, J.; Vanderfaeillie, J.; Vercruysse, N.; Tafforeau, J.; Van Oyen, H.; Vandevijvere, S. No association between elevated thyroid-stimulating hormone at birth and parent-reported problem behavior at preschool age. *Front. Endocrinol.* **2016**, *7*, 161. [CrossRef]
40. Trumpff, C.; De Schepper, J.; Vanderfaeillie, J.; Vercruysse, N.; Van Oyen, H.; Moreno-Reyes, R.; Tafforeau, J.; Vandevijvere, S. Neonatal thyroid-stimulating hormone concentration and psychomotor development at preschool age. *Arch. Dis. Child.* **2016**, *101*, 1100–1106. [CrossRef]
41. West, R.; Hong, J.; Derraik, J.G.B.; Webster, D.; Heather, N.L.; Hofman, P.L. Newborn Screening TSH Values Less Than 15 mIU/L Are Not Associated with Long-term Hypothyroidism or Cognitive Impairment. *J. Clin. Endocrinol. Metab.* **2020**, *105*, dgaa415. [CrossRef]
42. Freire, C.; Ramos, R.; Amaya, E.; Fernández, M.F.; Santiago-Fernández, P.; Lopez-Espinosa, M.J.; Arrebola, J.P.; Olea, N. Newborn TSH concentration and its association with cognitive development in healthy boys. *Eur. J. Endocrinol.* **2010**, *163*, 901–909. [CrossRef] [PubMed]
43. Lain, S.J.; Wiley, V.; Jack, M.; Martin, A.J.; Wilcken, B.; Nassar, N. Association of elevated neonatal thyroid-stimulating hormone levels with school performance and stimulant prescription for attention deficit hyperactivity disorder in childhood. *Eur. J. Pediatr.* **2021**, *180*, 1073–1080. [CrossRef] [PubMed]
44. de Andrade, J.E.; Dias, V.M.A.; Jardim de Paula, J.; Silva, I.N. Socioeconomic aspects are crucial to better intellectual outcome in early-treated adolescents with congenital hypothyroidism. *Child. Neuropsychol.* **2021**, *27*, 587–600. [CrossRef] [PubMed]

45. Pelaez, J.M.; Rojas-Ramos, J.C.R.; Domingos, M.T.; de Lima, M.R.; Kraemer, G.C.; Cardoso-Demartini, A.A.; Pereira, R.M.; Lacerda, L.D.; Nesi-França, S. Cognitive outcome of 458 children over 25 years of neonatal screening for congenital hypothyroidism. *J. Pediatr.* **2023**, *99*, 478–484. [CrossRef] [PubMed]
46. Bongers-Schokking, J.J.; Resing, W.C.M.; Oostdijk, W.; de Rijke, Y.B.; Keizer-Schramaa, S.M.P.d.M. Relation between Early Over- and Undertreatment and Behavioural Problems in Preadolescent Children with Congenital Hypothyroidism. *Horm. Res. Paediatr.* **2019**, *90*, 247–256. [CrossRef] [PubMed]
47. Villanger, G.D.; Ystrom, E.; Engel, S.M.; Longnecker, M.P.; Pettersen, R.; Rowe, A.D.; Reichborn-Kjennerud, T.; Aase, H. Neonatal thyroid-stimulating hormone and association with attention-deficit/hyperactivity disorder. *Paediatr. Perinat. Epidemiol.* **2020**, *34*, 590–596. [CrossRef]
48. Bongers-Schokking, J.J.; Resing, W.C.M.; Oostdijk, W.; De Rijke, Y.B.; De Muinck Keizer-Schrama, S.M.P.F. Individualized treatment to optimize eventual cognitive outcome in congenital hypothyroidism. *Pediatr. Res.* **2016**, *80*, 816–823. [CrossRef]

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Managing Newborn Screening Repeat Collections for Sick and Preterm Neonates

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Abstract: Some preterm and sick neonates have altered biochemical profiles and follow-up newborn screening (NBS) collections are recommended. The Victorian NBS program historically recommended repeat collections for babies with birth weight < 1500 g (managed by the maternity service provider) and 3 weeks post-transfusion (managed by the laboratory). We aimed to determine adherence to current guidelines and review the guidelines to improve NBS performance. To do this, we audited data from 348,584 babies between January 2018 and June 2022. Babies with a recorded birth weight of <1500 g were filtered for inclusion. For the overall review and visualization of the protocol, we sourced information from the literature, our professional society and tertiary hospital services. A total of 2647 babies had a birth weight recorded between 200 and 1499 g. Of these, 2036 (77%) had a second sample collected, indicating that >1 in 5 babies were not receiving a follow-up collection. Our timing of repeat collections for transfused babies, requiring a 3-week follow-up collection, was longer than in other Australasian jurisdictions. A new combined "sick–prem protocol" was launched to support repeat collections and after a 1-year review achieved 95% compliance. We recommend NBS laboratories audit preterm and sick neonate repeat collections to ensure appropriate follow-up. This should be supported with a visual process map to aid education and compliance.

Keywords: prematurity; low birth weight; transfusion; sick neonate; quality indicators; congenital hypothyroidism; transient hypothyroidism; congenital adrenal hyperplasia

1. Introduction

Low birth weight (LBW), preterm and sick neonates are a small but complicated cohort for newborn screening (NBS) programs. They require additional consideration and protocols to ensure they are appropriately screened [1,2]. The management of this cohort in our jurisdiction (Victoria, Australia) has traditionally been by the maternity service providers for the LBW babies following our repeat collection guideline, and for sick babies requiring a blood (red blood cell) transfusion this was managed by the NBS laboratory team. Some babies are both sick and LBW and this is complicated for the health care providers in the ward and the laboratory. Clear guidance to facilitate repeat collections is therefore needed. However, for this special cohort of neonates, there are gaps in evidence-based best practices to inform the re-sampling requirements.

Neonatology is a young discipline that has developed in parallel over the same period as NBS. Today neonatologists are very good at keeping preterm (20 to <37 weeks gestation) babies alive. This cohort now makes up about 8.2% of births in Australia and

globally ranges from 4 to 16% of all births. A subset of these includes very and extremely preterm or LBW births [3,4]. This improvement in care coincides with the increased number of preterm babies needing follow-up by NBS programs [3,5]. LBW (<2500 g) can be caused by intrauterine growth restriction, prematurity or both, and therefore because LBW considerably overlaps with preterm birth, and its measurement is generally considered more reliable than gestational age (GA), it has been used as the primary decision point for all preterm repeat collections in Australasia [6,7]. We note, though, that being born too small is conceptually different from being born too early. Irrespective, it is the very preterm (28 to < 32 weeks), extremely preterm (<28 weeks) and very LBW neonates that provide the significant challenges for improving morbidity and interpreting laboratory test results. In addition, the sick neonate who requires a transfusion of blood products further challenges NBS programs to ensure false negative screening results do not occur.

Certainly, the smaller the baby, the more immature the organ systems are, and in the case of the endocrine systems this can result in false-negative NBS results for thyroid screening. Thyroid hormone levels are known to decrease in the first week of life and probably reflect a transient depletion of thyroid hormone reserves. This nadir is more pronounced in preterm neonates, especially in infants <30 weeks gestation [8]. This also coincides with an immature hypothalamic–pituitary axis where the ability to produce TSH in response to low T4 and T3 is blunted [9]. As such, the first measurement of TSH in the usual timeframe of 48–72 h of life may miss cases of congenital hypothyroidism (i.e., false negative); and conversely, if thyroxine is the first tier measurement, false positive cases may occur [10]. Likewise, preterm babies have a persistent fetal adrenal zone and therefore some also require a follow-up collection when screening for congenital adrenal hyperplasia (CAH) [11,12]. Hence the need and routine recommendation for repeat collection(s) in these instances.

With the introduction of an increased number of conditions into NBS programs globally, the complexities of managing the preterm and sick neonates across all conditions are challenging. Recently we introduced CAH screening, with other conditions planned for introduction in quick succession [13]. With more conditions come some compromises in timing of repeat collections to avoid excess sample collections and iatrogenic anemia. Given the complexities involved, we had previously received requests from our maternity service providers for a visualization of the repeat collection cascade for preterm and sick neonates. Hence, it was timely to review (audit) our processes and ideally set up a system to manage repeat collections for preterm and sick neonates that was robust, evidence-based and harmonized with other Australasian programs.

We therefore aimed to (a) determine adherence to our current guidelines; and (b) review and revise (if required) the guidelines with visualization to improve NBS performance.

2. Materials and Methods

2.1. Victorian NBS Process at Time of Audit Initiation

The Victorian Newborn Screening program services approximately 80,000 babies born annually. On arrival at the laboratory, one 3.2 mm spot is punched from the card for each first-tier assay. Commercial kits are run for TSH, 17 hydroxy progesterone (17OHP) and immunoreactive trypsin (IRT) on the Genetic Screening Processor (GSP®) from Revvity (Turku, Finland). The first-tier metabolic screening of amino acids and acyl carnitines is run by an in-house method on Waters Xevo mass spectrometry instruments. Spinal muscular atrophy (SMA) and severe combined immunodeficiency (SCID) by a hyper-automated PCR procedure were being prepared for introduction in 2023. Other conditions planned for implementation are galactosemia, X-linked adrenoleukodystrophy and sickle cell disease screening in 2024, 2025 and 2026, respectively.

At the time of the audit (in 2022), the Victorian NBS program collection guideline recommended routine collections for:

- All babies—between 48 and 72 h of life. From 2020, i.e., the start of the COVID-19 pandemic, samples were accepted down to 36 h of age without the need for a repeat collection.
- Low birthweight babies—babies with a birth weight < 1500 g required a regular collection and then a follow-up collection at 2 weeks (1000–<1500 g at birth) or 3 weeks (<1000 g at birth) of age. Organization of the repeat samples was managed independently by the maternity service provider without prompting from the NBS laboratory. The NBS laboratory was only involved as needed to prompt follow-up of borderline or screen positive results. Therefore, if the repeat collection did not happen because it was missed by the maternity service provider, the NBS lab did not have a role in recognizing this.
- Transfused neonates—babies who received any form of transfusion prior to the routine NBS collection, required an early sample (pre-transfusion), a 48 h post-transfusion, plus a 3-weeks post-transfusion. These repeat collections were managed by the NBS laboratory; i.e., where electronic letters were sent out to remind providers to perform the repeat collection and follow-up of outstanding samples.

2.2. Audit of Repeat Collection Guideline for Preterm Neonates

Babies with first samples received between January 2018 and June 2022 were included in the review. Babies with a recorded birth weight of <1500 g were filtered for inclusion as the denominator and the number of these babies that had more than one dried blood spot (DBS) collection was used as the numerator to give a percentage of preterm babies with a follow-up collection. As this was a quality improvement audit and part of the routine continual improvement management practices of the NBS program, ethical approval was not required.

2.3. Review of Evidence and Harmonization

For the overall review and visualization of the protocol, we sourced information from the literature and professional bodies.

- The USA's Clinical Laboratory Standards Institute (CLSI) document NBS03 was extensively reviewed as part of the literature and review looking for their recommendations and the evidence to support this [1].
- The Human Genetics Society of Australasia NBS Committee was the professional body consulted, and each of the five other jurisdictions was asked (1) what their current recommendations were for preterm and transfused neonates, and (2) what is the evidence for their recommendation [14]?
- The World Health Organization definitions for low birthweight [15] and prematurity [16] were used to compare with other recommendations—Table 1.

Table 1. WHO definitions for low birthweight [15] and prematurity [16].

Birthweight (BW)	Preterm (PT) by Gestational Age (GA)
• Low BW < 2500 g	• Moderate or late PT 32–<37 weeks
• Very Low BW < 1500 g	• Very PT 28–<32 weeks
• Extremely Low BW < 1000 g	• Extremely PT < 28 weeks

2.4. New Protocol Design

The initial protocol design was performed in Microsoft PowerPoint, with changes recorded as new individual slides with dates and reason for change noted. Input was also invited from Victorian tertiary hospital services that had special care nurseries.

2.5. One Year On—Review of Impact of New Protocol

One year of data (May 2023 to April 2024) was retrieved from our laboratory information management system to review whether the change to the preterm protocol had

resulted in improved second sample collection. Qualitative feedback was sought from the laboratory team on the effect of this change on their work. Anecdotal feedback was sought from the maternity service providers via our NBS Educator and review of our management system for instances of non-compliance.

2.6. Statistical Analysis

The patient data were exported from our laboratory information system into a CSV file in Microsoft Excel. Data were sorted based on birth weight (and gestational age for the updated SP protocol only) for inclusion and a simple count was performed to show if these babies had received at least a second collection. Descriptive calculations were performed in Microsoft Excel and Stata-18.0. Q-Pulse (Ideagen, Nottinghamshire, UK) was used to manage the audit.

3. Results

3.1. Audit of Current Repeat Collection Guideline for Preterm Neonates

A total of 348,584 NBS babies were screened between January 2018 and June 2022. Of these, 2647 babies had a birth weight recorded between 200 and 1499 g. From this subset, 2036 (77%) had a second sample collected, indicating that >1 in 5 babies were not receiving a follow-up collection.

3.2. Review of Evidence and Harmonization

The CLSI document was extensively reviewed, and this provided some recommendations for repeat collections. This included that transfusions were specifically whole blood transfusions and that extra corporeal membrane oxygenation (ECMO) babies should be included [1]. Representative(s) from all six Australasian jurisdictions were present at the HGSA Newborn Screening Committee meeting and responded to the questions that formed the information provided regarding the transfusion protocol. The information on LBW repeat collections was previously collated as part of a publication on harmonization of congenital hypothyroidism screening [7] (Table 2).

The consensus was that the evidence for the collection time frame for transfusions was not clear. Our Victorian time of repeat collections for transfused babies, requiring a 3-week follow-up collection, was longer than that in other Australasian jurisdictions, i.e., ours was at 3 weeks whereas others were either 1 or 2 weeks post-transfusion.

Table 2. Comparison of time of repeat collection guideline per jurisdiction for preterm and sick neonates [1]. Note: all jurisdictions recommended a pre-transfusion sample for affected babies.

Jurisdiction	Preterm—Second Sample, Adapted from [7]	Post-Transfusion Sample
NSW	• <1500 g or <30/40 weeks repeat at one month	48 h + 2 weeks
QLD	• <1500 g repeat screen 14 days • <1000 g repeat screen again 28 days	48 h + 2 weeks
SA	• <1500 g repeat at 10 days and again at 30 days (or at discharge)	1 week
VIC	• 1000–<1500 g repeat at 2 weeks • <1000 g repeat at 3 weeks	48 h + 3 weeks
WA	• ≤1500 g repeat screen 14 days • ≤1000 g repeat screen again 28 days	48 h
NZ	• ≤1500 g repeat screen 14 days • ≤1000 g repeat screen again 28 days	1 week
CLSI 2009 [1] *	• <34 weeks GA or <2000 g repeat screen for thyroid and CAH and other unresolved tests at 28 days of age	120 days *

Table 2. *Cont.*

Jurisdiction	Preterm—Second Sample, Adapted from [7]	Post-Transfusion Sample
AAP 2023 [2] **	• <32 weeks GA or <1500 g repeat screen for thyroid at 2–4 weeks of age • Plus, further follow-up if baby has not reached 36 weeks corrected GA at time of second sample	2–4 weeks of age

[1] HGSA meeting discussion—v3 new protocol and comparison—meeting 2 March 2023. The five jurisdictions in Australia are New South Wales (NSW), Queensland (QLD), South Australia (SA), Victoria (VIC), and Western Australia (WA). There is only one screening service for New Zealand (NZ). * This recommendation considers an extended screening panel that includes hemoglobinopathies. ** Recommendations specific for thyroid screening, published post our audit.

3.3. New Sick–Prem (SP) Protocol

A new combined "sick–prem protocol" was launched (17 April 2023) to support repeat collections (see Figure 1). Under this protocol, all sick and preterm neonatal repeat collections are now managed by the NBS laboratory. At the time of data entry, any sample that meets the criteria has an "SP" flag added to the sample. For transfusions, the SP flag is added, and we also add the specific transfusion flag "Tx". This initiates an electronic repeat collection letter to be generated and sent from the laboratory information management system. Follow-up reminder letters are also scheduled using this electronic process. If a repeat collection sample is not received, our NBS nurse educator phones the relevant maternity service provider to follow up. For the preterm neonates, the NBS laboratory closes the repeat collection request (for babies with screen negative results) once a sample has been received by at least 3 weeks postnatal age. With this new protocol, it was decided to advise maternity service providers of this broader timeline of 36 to 72 h for routine collections as part of the updated collection guideline [17].

3.4. Adherence to New SP Protocol

In the year from May 2023 to April 2024 inclusive, 76,403 babies were screened. Of these, 668 babies (0.9%) had a recorded birth weight (BW) and/or gestational age (GA) that met the SP criteria (Table 3).

Table 3. One-year follow-up audit of SP protocol (May 2023 to April 2024) showing the n = 668 babies who met the inclusion criteria of GA < 32 weeks and/or BW < 1500 g. Data are shown divided into specific subsets of the inclusion criteria to demonstrate the makeup of this cohort. Overall, 95% had a second sample collected.

SP Criteria	First Sample	Second Sample	Percentage of Second Samples
GA < 32 weeks	566	546	96%
BW < 1500 g	635	606	95%
Both BW and GA	523	507	97%
GA < 32 weeks and no BW	4	N/A	N/A
BW < 1500 g and no GA	333	N/A	N/A

Figure 1. Revised newborn screening visual process map for sick and preterm neonates in Victoria Australia; effective from Monday 17 April 2023. Follow-up collections occur outside of this protocol for NBS results deemed screen-positive, i.e., requiring a re-collection or referral to a clinical team.

This had a small organizational impact on the newborn screening program delivery for this small number (<1%) of babies. The organization impact tool allows an assessment of the impact of the change on other aspects of the process and includes here the impact on the baby, the changes to reporting processes and the workload for the various teams involved in NBS from collection through to the clinical follow-up. Details on organizational impact can be found at [18] (Figure 2).

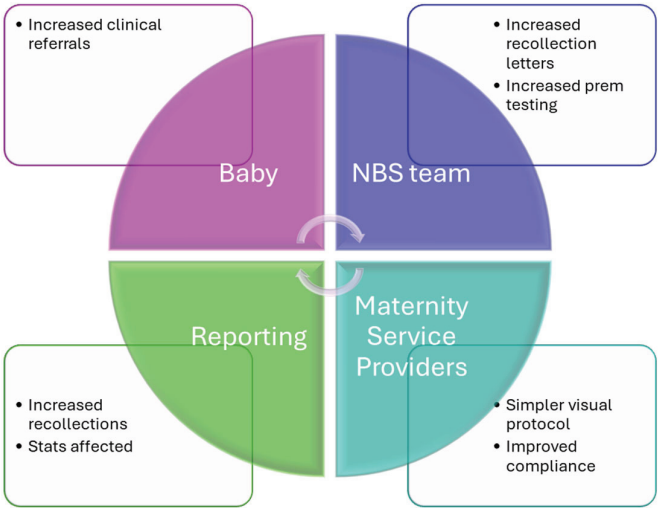

Figure 2. Organizational impact of SP protocol—protocol live from Monday 17 April 2023 [18]. We estimated about 160 babies in our jurisdiction received the additional collection over the 12-month follow-up audit period (May 2023 to April 2024).

4. Discussion

With the introduction of an increased number of conditions, and requests for a flow-diagram visualization of a combined protocol for preterm and sick neonates, we looked to undertake a full review of our current processes. This led to the successful introduction of our new sick–prem (SP) protocol, which is provided in Figure 1. The successful completion of our preterm and sick repeat collection audit has resulted in improved and clearer processes for the management of this cohort. This is supported by the increase in the previous 76% adherence to now achieving 95% collection of second samples in this cohort. It also demonstrates the importance of data-driven review for continuous improvement.

Screening for primary congenital hypothyroidism commenced in Victoria and all of Australasia in the 1970s [7,19–21]. At this time, the mortality for extremely preterm infants was high; however, with subsequent improvements in survival, consideration turned to improving morbidity and studies of thyroid function demonstrated differences in preterm infants compared to full-term neonates [10]. In preterm neonates, thyroxine levels are known to decrease in the first few weeks of life and the difference between preterm and full-term neonates persists until 30–49 days postnatally [22]. In a study of preterm neonates <30 weeks gestation conducted by our group a decade ago, we confirmed that serum-free thyroxine levels were as low as <2.6 pmol/L for the first 28 days with the nadir at 7 days. We also identified differences in the thyroid hormone concentration in the younger (23–26 weeks) compared to the older (27–29 weeks) preterm babies; i.e., fT4 levels were significantly lower in the younger group compared to the older preterm neonates [23]. Furthermore, the condition of transient hypothyroxinemia of prematurity (THOP) is now established and affects infants born at less than 30 weeks gestation. THOP is characterized by an initial rise in T4 at 24 h post-birth followed by a decline compared to cord blood levels with a nadir at 7 days of age, and TSH secretion at this time sees a return of T4 levels; however, in the sickest preterm neonates, this 24 h surge is often absent. An association also exists between THOP and adverse outcomes among the preterm neonates and this association is strongest for babies born at less than 28 weeks gestation [22]. We compared this combined evidence with the CLSI recommendations for timing of follow-up collection "at 28 days of age or discharge, whichever comes first" [1]. This CLSI advice was the basis of our timing for follow-up collections in our preterm algorithm given the research-based evidence found on the time the thyroid axis typically starts behaving as expected for a full-term neonate. Following the update of our protocol, the AAP guideline for CH screening was released and it is concordant with our decision of retesting babies who at birth were <1500 g and/or <32 weeks GA [2].

Very or extremely low birth weight has been used as the decision point for our program, but some other programs also include gestational age-related decision points. On reviewing the CLSI guideline cutoffs of 2000 g and 34 weeks for rescreening of thyroid panel and CAH, we noted these did not coincide with the definitions of the WHO [1,15,16]. Across Australasia, repeat screening is harmonized to babies with a birthweight of <1500 g, which is consistent with others [2,7,24]. With a view to staying harmonized with other Australasian NBS programs, and consistent with current neonatal definitions, we decided to stay with the <1500 g cut point for the routine repeat collection [15]. However, during the audit, we noted a few babies who had a GA recorded without a birthweight and given the recommendation in the CLSI guideline to include GA, we added a GA < 32 weeks for follow-up collection; i.e., the WHO cut point for very preterm [16]. The addition of GA only captured an extra 5% of babies and therefore the inclusion of this extra parameter does not significantly influence the number of repeat collections required. Overall, our decisions here are around harmonization as the evidence for appropriate cut points becomes a compromise between all the conditions screened and clinical risk.

The evidence around the decision and timing to recollect for transfusions was scant. In our jurisdiction, we had been performing a transfusion protocol for all babies that had had any form of transfusion. On reviewing the CLSI guideline, the focus was on red blood cell (RBC) transfusions and extracorporeal life support (ECLS), indicating that a RBC transfusion invalidates multiple tests for newborn screening [1]. Hence, using the advice in this guideline, we pulled back to only requesting transfusion repeat collections related to babies who had received RBC or ECLS. We then consulted with the HGSA NBS Committee on the rationale for repeat collections, especially timing, and we found our practice extended longer than in other jurisdictions, and while there was limited evidence provided for the timing of follow-up collections, we moved to harmonize with the other jurisdictions in Australasia and changed from 3 weeks to 1 week post-transfusion for a follow-up collection. We note, however, that further transfusion-related repeat collections may need to be considered in association with the proposed introduction of sickle cell screening [25].

The change to the timing of collection was implemented at the start of the COVID-19 pandemic in 2020 and formalized for all babies as part of this SP introduction [26]. This was carried out in the background using evidence from New Zealand and Western Australia. The aim of this change was to screen the babies before leaving the hospital. Overall, our data indicates that results in the window of 36–72 h are consistent with the traditional window of 48–72 h, and with the trend of earlier discharge from hospital this change has been seen as an advantage to the maternity service providers' workflow. Notably, other programs in Australasia have a similar expanded window, but continue to state 48–72 h on their information [7]. Advertising this change did result in feedback related to the hospital's ability to be agile with updating in-house documentation (which stated 48–72 h), but as this timeframe was only extended, the hospital's documentation could be updated with the next review cycle.

There were a few limitations to this study and protocol review. The first one relates to the design of the original preterm baby second collection review, which looked at only the receipt of a second collection and not the actual timing of the second sample. This could mean that the overall adherence rate could be lower than the 76% of babies identified in this study. The second limitation relates to the unknown survival rate of this cohort, and this may have influenced the repeat collection numbers. Finally, while this review was performed in conjunction with the implementation of CAH screening, the information provided in the CLSI guideline recommended 4 weeks post-birth for a follow-up collection. However, it is well established that the fetal adrenal gland persists until at least the equivalent of term despite early delivery. Hence, 4 weeks for extreme preterm neonates will not approach this milestone of full-term equivalent at 4 weeks of age and the evidence of ontogeny suggests that a longer time period for follow-up may be warranted. To date, given the rate of classical CAH approximates 1:15,000 and all pre-terms

make up 8.2% of births, we have not yet identified a preterm baby with CAH and such babies identified should inform future practice. In the absence of evidence, harmonization of practice is the best we can achieve to ensure all babies, irrespective of the jurisdiction, receive the same care.

Overall, the implementation of this preterm protocol has led to a significant change that has in turn impacted the organization of our NBS program. In the pre-analytical phase, the higher compliance with repeat collections indicates successful integration of the new protocol across maternity provider services in our jurisdiction. The higher workload involved for staff who perform the repeat tests is not new, however, rather more consistently recognized and completed compared to pre-2023. However, the NBS laboratory team now need to manage and follow up the maternity service providers that fail to send in a timely repeat collection, and this has added to the overall workload. Post-analytically, more babies are likely to be referred and so the clinical teams will potentially be assessing more babies, but the sample size is too small to adequately review the impact currently. The benefit to the baby in doing more good than harm is paramount for our NBS program and the design of the SP protocol process map allows for easy change as we continue to review our processes and impact into the future.

5. Conclusions

We recommend NBS laboratories audit preterm and sick neonate repeat collections to ensure appropriate follow-up of babies. This should be supported with a visual process map to aid education and compliance.

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Institutional Review Board Statement: This was a continuous improvement review of the newborn screening program that was conducted post the implementation of CAH screening. As this was a quality improvement activity, institutional ethical approval was not required.

Informed Consent Statement: All samples tested after informed consent of the parent/caregiver as part of the newborn screening program process.

Data Availability Statement: Please email the corresponding author if you would like to discuss the background data further.

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References

1. Clinical and Laboratory Standards Institute. *Newborn Screening for Preterm, Low Birth Weight and Sick Newborns*, 2nd ed.; CLSI Guideline NBS03; CLSI: Wayne, PA, USA, 2019; p. 82.
2. Rose, S.R.; Wassner, A.J.; Wintergerst, K.A.; Yayah-Jones, N.H.; Hopkin, R.J.; Chuang, J.; Smith, J.R.; Abell, K.; LaFranchi, S.H. Congenital Hypothyroidism: Screening and Management. *Pediatrics* **2023**, *151*, e2022060419. [CrossRef] [PubMed]
3. World Health Organization (WHO). *Preterm Birth*; World Health Organization: Geneva, Switzerland, 2020.
4. *Australian Institute of Health and Welfare (AIHW) Australia's Mothers and Babies*; AIHW: Canberra, Australia, 2023.

5. Australian Institute of Health and Welfare (AIHW). *Preliminary Data Tables: National Perinatal Data Collection Annual Update 2022*; Australian Institute of Health and Welfare, Ed.; Australian Government: Canberra, Australia, 2022.
6. UNICEF; WHO. *Country Consultation on Low Birthweight and Preterm Birth Estimates—Technical Note*; WHO: Geneva, Switzerland, 2022.
7. Huynh, T.; Greaves, R.; Mawad, N.; Greed, L.; Wotton, T.; Wiley, V.; Ranieri, E.; Rankin, W.; Ungerer, J.; Price, R.; et al. Fifty years of newborn screening for congenital hypothyroidism: Current status in Australasia and the case for harmonisation. *Clin. Chem. Lab. Med.* **2022**, *60*, 1551–1561. [CrossRef] [PubMed]
8. Van Wassenaer, A.G.; Kok, J.H.; Dekker, F.W.; de Vijlder, J.J. Thyroid function in very preterm infants: Influences of gestational age and disease. *Pediatr. Res.* **1997**, *42*, 604–609. [CrossRef] [PubMed]
9. Mitchell, M.L.; Walraven, C.; Rojas, D.A.; McIntosh, K.F.; Hermos, R.J. Screening very-low-birthweight infants for congenital hypothyroidism. *Lancet* **1994**, *343*, 60–61. [CrossRef] [PubMed]
10. Nolan, B.; Uy, C.; Stablein, L.; Bany-Mohammed, F. Screening for Delayed Thyroid Stimulation Hormone Rise and Atypical Congenital Hypothyroidism in Infants Born Very Preterm and Infants with Very Low Birth Weight. *J. Pediatr.* **2024**, *269*, 113974. [CrossRef] [PubMed]
11. Midgley, P.C.; Russell, K.; Oates, N.; Shaw, J.C.; Honour, J.W. Activity of the adrenal fetal zone in preterm infants continues to term. *Endocr. Res.* **1996**, *22*, 729–733. [CrossRef] [PubMed]
12. Greaves, R.F.; Jevalikar, G.; Hewitt, J.K.; Zacharin, M.R. A guide to understanding the steroid pathway: New insights and diagnostic implications. *Clin. Biochem.* **2014**, *47*, 5–15. [CrossRef] [PubMed]
13. Greaves, R.F.; Kumar, M.; Mawad, N.; Francescon, A.; Le, C.; O'Connell, M.; Chi, J.; Pitt, J. Best Practice for Identification of Classical 21-Hydroxylase Deficiency Should Include 21 Deoxycortisol Analysis with Appropriate Isomeric Steroid Separation. *Int. J. Neonatal Screen.* **2023**, *9*, 58. [CrossRef] [PubMed]
14. Human Genetics Society of Australasia (HGSA). *Newborn Screening Committee*; HGSA: Mascot, NSW, Australia, 2024.
15. Cutland, C.L.; Lackritz, E.M.; Mallett-Moore, T.; Bardají, A.; Chandrasekaran, R.; Lahariya, C.; Nisar, M.I.; Tapia, M.D.; Pathirana, J.; Kochhar, S.; et al. Low birth weight: Case definition & guidelines for data collection, analysis, and presentation of maternal immunization safety data. *Vaccine* **2017**, *35 Pt A*, 6492–6500. [CrossRef]
16. Ohuma, E.O.; Moller, A.B.; Bradley, E.; Chakwera, S.; Hussain-Alkhateeb, L.; Lewin, A.; Okwaraji, Y.B.; Mahanani, W.R.; Johansson, E.W.; Lavin, T.; et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: A systematic analysis. *Lancet* **2023**, *402*, 1261–1271. [CrossRef] [PubMed]
17. Victorian Newborn Bloodspot Screening Program. *Newborn Bloodspot Screening Comprehensive Collection Guideline*, 4th ed.; VCGS, Ed.; Victorian Clinical Genetics Services (VCGS): Parkville, Australia, 2021.
18. Greaves, R.; Kricka, L.; Gruson, D.; Ferrari, M.; Martin, H.; Loh, T.P.; Bernardini, S. Toolkit for emerging technologies in laboratory medicine. *Clin. Chem. Lab. Med.* **2023**, *61*, 2102–2114. [CrossRef] [PubMed]
19. Connelly, J.F.; Coakley, J.C.; Gold, H.; Francis, I.; Mathur, K.S.; Rickards, A.L.; Price, G.J.; Halliday, J.L.; Wolfe, R. Newborn screening for congenital hypothyroidism, Victoria, Australia, 1977–1997. Part 1: The screening programme, demography, baseline perinatal data and diagnostic classification. *J. Pediatr. Endocrinol. Metab. JPEM* **2001**, *14*, 1597–1610. [CrossRef] [PubMed]
20. Connelly, J.F.; Rickards, A.L.; Coakley, J.C.; Price, G.J.; Francis, I.; Mathur, K.S.; Wolfe, R. Newborn screening for congenital hypothyroidism, Victoria, Australia, 1977–1997. Part 2: Treatment, progress and outcome. *J. Pediatr. Endocrinol. Metab. JPEM* **2001**, *14*, 1611–1634. [CrossRef] [PubMed]
21. Coakley, J.C.; Francis, I.; Gold, H.; Mathur, K.; Connelly, J.F. Transient primary hypothyroidism in the newborn: Experience of the Victorian Neonatal Thyroid Screening Programme. *Aust. Paediatr. J.* **1989**, *25*, 25–30. [CrossRef] [PubMed]
22. Mercado, M.; Yu, V.Y.; Francis, I.; Szymonowicz, W.; Gold, H. Thyroid function in very preterm infants. *Early Hum. Dev.* **1988**, *16*, 131–141. [CrossRef] [PubMed]
23. Greaves, R.F.; Zacharin, M.R.; Donath, S.M.; Inder, T.E.; Doyle, L.W.; Hunt, R.W. Establishment of hormone reference intervals for infants born <30 weeks' gestation. *Clin. Biochem.* **2014**, *47*, 101–108. [PubMed]
24. Ares Segura, S.; Casano-Sancho, P.; Chueca Guindulain, M. Assessment of thyroid function in the preterm and/or very low birth weight newborn. *An. De Pediatría (Engl. Ed.)* **2021**, *95*, 277.e1–277.e8. [CrossRef] [PubMed]
25. *Newborn Bloodspot Screening: What is Screened in the Program*; Department of Health and Aged Care, Australian Government: Canberra, Australia, 2024.
26. Greaves, R.F.; Pitt, J.; McGregor, C.; Wall, M.; Christodoulou, J. Newborn bloodspot screening in the time of COVID-19. *Genet. Med.* **2021**, *23*, 1143–1150. [CrossRef] [PubMed]

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Screening Blind Spot: Missing Preterm Infants in the Detection of Congenital Hypothyroidism

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Abstract: Congenital hypothyroidism (CH) is a critical condition in infancy where early detection is vital for optimal development. This study aimed to evaluate the sensitivity of Aotearoa New Zealand's Newborn Metabolic Screening "Low Birth Weight" protocol for detecting CH in preterm infants. A 10-year audit was conducted on 2935 preterm infants (<2000 g or ≤34 weeks gestation) screened within NICUs or SCBUs in the Auckland region. The study assessed both screen-detected and clinically detected cases of CH. Data were collected from screening and clinical records to evaluate the sensitivity and reliability of the current protocol. The audit identified 19 cases of primary CH, with a 1:154 incidence. Thirteen cases met the criteria for inclusion in the audit. Just over half of the eligible cases (7/13) were screen-detected, while the remaining were detected clinically, suggesting limitations in screening sensitivity. The analysis revealed that the protocol missed permanent as well as transient cases, and that biochemical severity was not predictive of permanence. A revised screening protocol was developed and commenced in July 2024.

Keywords: congenital hypothyroidism; preterm; screening; newborn screening; low birth weight

1. Introduction

Primary congenital hypothyroidism (CH) is the most common disorder detected on newborn metabolic (bloodspot) screening and a common preventable cause of intellectual disability worldwide [1–3]. Primary CH causes thyroid hormone deficiency due to an absent, ectopic, or under-functioning thyroid gland. While the overall incidence of CH is approximately 1:2000, it is much higher among preterm infants, with reported rates ranging from 1:100 to 1:150 in very preterm infants [2,4–8]. Whilst CH in preterm babies is frequently transient, a significant proportion of preterm infants have permanent CH [7] and thyroid-stimulating hormone (TSH) concentrations at diagnosis do not appear to be a reliable predictor of permanence [9]. The aetiology of the increased frequency of CH in preterm infants remains unclear but is hypothesised to be due to several factors, including immaturity of the hypothalamic–pituitary–thyroid axis, medications (steroids, dopamine), non-thyroidal illness (e.g., respiratory distress syndrome), and iodine deficiency [5,8,10,11]. The maturation of thyroid function in preterm infants differs from that of term infants, necessitating a revised screening protocol in this population, which is discussed in detail elsewhere [7,8].

In Aotearoa New Zealand, nearly 8% of infants are born preterm (before 37 weeks of gestation) [12]. Prematurity is the most common reason for admission to a level III neonatal unit, with very preterm babies (<32 weeks gestation) accounting for 25–30% of admissions. Māori and Pasifika infants are over-represented in the level III neonatal unit, making up 21.3% and 11.8% of admitted infants, respectively, compared to their proportion of 16.5% and 8.5% in the general population [13,14].

Over the past decade, screening programmes have become increasingly aware that preterm infants with CH are likely to have normal initial screening results due to a delayed rise in TSH concentrations [15,16]. To improve the reliability of CH screening, the New Zealand screening programme introduced a protocol ('the Low Birth Weight (LBW) protocol') for repeat sample collection from low birth weight (<1500 g) infants in 2007. All participating infants had their first screening sample at 48–72 h of age, infants with a low birth weight of <1500 g had a second screening sample at 14 days of age, and extremely low birth weight infants (<1000 g) had a third screening sample at 28 days of age. Despite over 10 years of experience, the performance of the LBW protocol has never been comprehensively evaluated.

Our objective was to evaluate the screening sensitivity of this protocol by auditing both the screening and clinically detected primary CH diagnoses in preterm infants in the Auckland region over a 10-year period. Other aspects such as screening specificity, aetiology, and disease severity were out of the scope of this study.

2. Materials and Methods

2.1. Aotearoa New Zealand Newborn Metabolic Screening Process for Low Birth Weight Infants (<1500 g) During Audit Timeframe

Approximately 60,000 infants undergo Newborn Metabolic Screening in New Zealand each year, with very high (>99%) population coverage. Infants born with a birth weight of less than 1500 g followed the "Low Birth Weight Protocol", with approximately 500 infants screened nationwide under this protocol annually. The protocol included an initial screening at 48–72 h, a second at 14 days for infants <1500 g, and a third sample at 28 days for those born <1000 g (see Figure 1) [17]. Blood samples were collected onto specialised collection paper and sent to the single national screening laboratory at LabPlus, Auckland, New Zealand. Screening samples were analysed for markers of a full metabolic screening panel [17], including whole blood TSH concentration via AutoDELPHIA (Perkin-Elmer, now Revvity). Results were reported on the next working day after the sample was received.

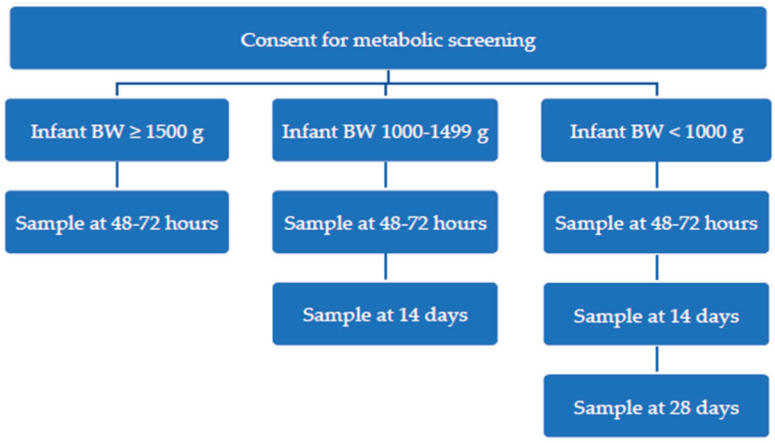

Figure 1. New Zealand newborn screening algorithm for congenital hypothyroidism during audit timeframe; BW = birth weight.

Elevated TSH results prompt notification to the responsible neonatologist for serum thyroid function tests. TSH thresholds for screening vary by postnatal age due to the physiological TSH surge immediately after birth, which rapidly declines over the first few days of life (Table 1).

Table 1. Newborn screening TSH thresholds during the audit timeframe.

Protocol prior to 1 January 2013:	
	TSH < 15 mIU/L = Normal TSH ≥ 15 mIU/L = Follow-up specimen required
Protocol from 1 January 2013:	
Age ≤ 14 days:	TSH < 15 mIU/L = Normal TSH ≥ 15 mIU/L = Follow-up specimen required
Age > 14 days:	TSH < 8 mIU/L = Normal TSH ≥ 8 mIU/L = Follow-up specimen required

Blood spot TSH thresholds are given in whole blood units. A conversion factor of 2.2 is typically used to estimate serum units, based on an assumed haematocrit of 0.55. However, preterm and low birth weight infants often have much lower haematocrits, which can result in falsely elevated TSH results when using this standard conversion [18]. Thresholds have since been adjusted further due to changes in the assay used and refinement of thresholds by postnatal age.

2.2. Audit Methodology

The Auckland region has a population of 1.5 million people, with an annual birth rate of approximately 22,000 live births per year [14,19]. Very preterm infants are cared for at two neonatal intensive care units and one special care baby unit across the region, providing level III and level II care, respectively. Starship Child Health provides a single regional referral service for infants and children with endocrine conditions, including CH.

Infants participating in newborn screening born at or before 34 weeks of gestation (<34 + 1) with a birth weight of <2000 g in the Auckland region between 1 January 2009 and 31 December 2018 were included in the audit. Non-Auckland residents, those born >34 weeks gestation, and any infants who died during their admission were excluded from the audit. Gestational age and birth weight parameters were wider than standard preterm screening criteria (<32 weeks gestational age and/or <1500 g birth weight) to assess if standard parameters were adequately detecting affected infants.

Infants with screen-detected CH were identified from newborn screening records. The Starship Child Health Paediatric Endocrinology Department's Thyroid Clinic patient database was cross-referenced against the newborn screening database to identify additional infants being followed up for CH that were not identified by screening.

Data extraction was performed by the laboratories at each hospital site to identify any infant born at <34 + 1/40 gestation within the audit timeframe at all sites that ever had serum thyroid function tests performed during their admission. Any infant with a serum TSH of ≥10 mU/L at any time was cross-referenced with the newborn screening data, Clinical Portal, and the Starship Paediatric Endocrinology Department's patient database to identify any remaining infants with congenital hypothyroidism that were not detected by screening and lost to follow-up by the local service. The threshold of 10 mU/L for serum

TSH was selected as a consensus, as infants with a TSH persistently above this level should be investigated further, if not initiated on levothyroxine supplementation [20].

Clinical data for each identified infant were examined manually to evaluate the type and magnitude of thyroid dysfunction, method of diagnosis, investigations, co-morbidities, exposure to intravenous contrast or topical antiseptics containing iodine, type and duration of treatment, and outcomes.

For the purpose of the audit, a case was defined as an infant with biochemical results supporting a diagnosis of primary congenital hypothyroidism (persistently elevated TSH concentration) and was initiated on thyroxine treatment. Severe cases were defined as serum TSH level $\geq$ 50 mIU/L in diagnostic testing, and moderate CH was defined as a serum TSH level $\geq$ 20 mIU/L. Infants treated with thyroxine due to sick euthyroid or central hypothyroidism were excluded from the audit, as these conditions are not targeted through our newborn screening approach. We defined "clinically detected" as cases detected through serum thyroid function tests and unscheduled screening cards that would not have otherwise been detected by the screening programme protocol.

2.3. Statistical Analysis

All patient data were collated in a CSV sheet in Microsoft Excel. Data were sorted by confirmed diagnosis of CH and the detection method. A simple count was performed to identify infants detected by screening compared to infants detected clinically. Each individual data set was then examined manually to describe characteristics of cases.

3. Results

An audit indicates that 2935 babies weighing less than 2000 g or born at 34 weeks gestation or earlier were screened in NICUs or SCBUs in the Auckland region. Nineteen cases of primary CH were identified, leading to a CH incidence of 1 in 154.

Complete data were available in 13/19 cases, which formed the basis of our audit. These cases are summarised in Table 2. Just over half the CH cases (7/13) were screen-detected, and the remainder (6/13) were clinically detected, indicating limited sensitivity of the current screening approach. An additional six cases of primary CH (four screen-detected and two clinically detected) were excluded from the audit due to living outside of the Auckland region and having incomplete data available for review. No additional cases were identified by cross-referencing regional laboratory results or from the paediatric endocrinology patient database.

Table 2. Summary of audit findings.

Sex	Gestational Age at Birth (Weeks + Days)	Birth Weight (g)	Ethnicity	Screen Positive Sample	Reason for Thyroid Function Tests	Age at Diagnosis	Screening Whole Blood TSH (mIU/L)	At Diagnosis		Outcome (Permanent, Transient)	Age Ceased Thyroxine	Co-Morbidities
								Serum TSH (mU/L) Ref: 0.4–16 mU/L	Serum Free T4 (pmol/L) Ref: 10–40 pmol/L			
Clinically detected												
Male	28 + 3	830	Samoan	Fourth	Third screen borderline for amino acid breakdown disorder, fourth sample requested	6 weeks	8	18.2	17	Transient	2 years	Very preterm, ambiguous genitalia with imperforate anus, ELBW, Apnoea of prematurity, RDS, jaundice
Male	28 + 3	1350	Samoan	Third	Unscheduled third screening sample	5 weeks	18	33	6.2	Transient	2 years	Very premature, RDS, Apnoea of Prematurity, RoP
Female	29	980	New Zealand European		Maternal Hypothyroidism	6 weeks		39	12.5	Transient	2 years	Very preterm, RoP, AOP, RDS, jaundice,
Male	30	1600	Chinese		Feeding problems	12 weeks		11.9	14	Transient	18 months	Very preterm
Male	31	1490	Indian		Pituitary screening	8 weeks		19.7	17	Permanent		Very preterm, 45XO/46XY, Hypospadias
Female	32	1420	New Zealand European		Dysmorphism, macrocephaly, and seizures	6 weeks		10	15	Permanent		Moderate preterm, RDS, later diagnosed with GDD
Screening detected												
Female	24 + 3	650	New Zealand European	Third		5 weeks	14	41	9.6	Transient	2 years	Extreme preterm, ELBW, CLD, Apnoea of prematurity, RoP, PDA
Male	27 + 5	1105	Fijian	Second	Borderline second screen, third sample requested	4 weeks	74	>100	3.7	Transient	2.5 years	Extreme preterm, VLBW, RDS, CLD, left inguinal hernia, CMV
Male	28 + 6	900	Cook Island Māori	Second		3 weeks	13	39.9	12.7	Transient	2.5 years	Very preterm, ELBW, RDS, hypospadias with chordee
Female	29 + 5	720	Cook Island Māori	Second		3 weeks	78	138	6	Permanent		Very preterm, ELBW, RDS, CLD, Twin 2
Female	30 + 1	1455	Chinese	Second		4 weeks	22	49	9.9	Transient	5 years	Very preterm, VLBW, RDS, jaundice, maternal GDM
Female	30 + 5	1065	New Zealand Māori	Second		17 days	32	>100	3.2	Transient	3 years	Very preterm, VLBW, apnoea of prematurity, AOP, PDA
Female	32 + 1	1235	Samoan	Second		4 weeks	19	42.2	10.9	Transient	6 months	Moderate preterm, VLBW, RDS, SGA

AOP = anaemia of prematurity; CMV = cytomegalovirus; CLD = chronic lung disease; ELBW = extremely low birth weight; GDD = global developmental delay; GDM = gestational diabetes mellitus; PDA = patent ductus arteriosus; RDS = respiratory distress syndrome; RoP = retinopathy of prematurity; SGA = small for gestational age; VLBW = very low birth weight.

All screen-detected cases were identified from samples collected at 2 or 4 weeks, following normal bloodspot TSH concentrations at 48–72 h. The majority of CH cases had biochemically moderate to severe thyroid disease (TSH $\geq$ 20 mIU/L to $\geq$50 mIU/L, respectively) at presentation. Most cases (10/13) had transient disease and were able to cease thyroxine replacement between 2 and 3 years of age. Biochemical severity at diagnosis was not predictive of permanent disease. None of the cases had been exposed to intravenous contrast.

Clinically detected cases were typically older and had milder biochemical disease at detection than screen-detected cases. The reasons for clinical detection were varied and included additional unscheduled screening cards, primary CH incidentally detected through pituitary function testing, investigation of dysmorphisms, and feeding problems. As with screen-detected cases, biochemical severity was not predictive of permanent disease.

4. Discussion

The goal of newborn metabolic screening is to facilitate better outcomes through the early identification of congenital disorders. It is not possible to detect all cases through screening, and there are numerous factors (e.g., prematurity, intravenous nutrition, transfusions) that can increase the likelihood of false-negative and false-positive screen results in NICU babies. Newborn screening has been adapted to better suit the NICU population through the introduction of additional samples and modified analyte thresholds. The global consensus supports repeat testing for low-birth-weight infants; however, there is no agreed-upon schedule for the timing of the samples, and it varies around the world. The main arguments for and against repeated testing balance early detection and treatment versus the cost of extra testing and potentially unnecessary subsequent clinical intervention.

Repeat screening sample collections in NICU babies can increase disorder detection and reduce referrals for diagnostic testing. For preterm and low-birth-weight infants in New Zealand, a complete "newborn screen" is considered a series of related tests, rather than a combination of two or three individual screens. Collection algorithms are designed to be simple enough to follow, as the screening programme is unable to send reminders, and it is assumed that all expected bloodspot samples will be received. The previous protocol was based on low birth weight (<1500 g) as a proxy for prematurity, as when discussed in 2007, this was felt to be a more objective, readily available measure. However, combined birth weight and gestational age criteria are commonly utilised in existing NICU protocols, suggesting that gestational age could similarly be incorporated into the metabolic (bloodspot) screening protocol. Victoria, Australia, recently amended their preterm and sick infant protocol and added gestational age <32 weeks and/or birth weight <1500 g. The addition of gestational age captured an extra 5% of infants and is felt not to have a significant influence on the number of repeat samples required [21].

As expected, we observed a greater than 10-fold higher incidence of primary CH in very preterm babies compared with the overall population, and very poor reliability of the initial sample in detecting CH. It was reassuring to note that the most severe cases (TSH $\geq$ 50 mIU/L) were screen-detected through the current protocol, as a result of samples collected at either 2 or 4 weeks postnatally. A cluster of cases in very preterm babies who were just under or above the 1500 g birth weight threshold suggested that reliability may be increased by incorporating gestational age as an 'and/or' criteria with birth weight (i.e., a protocol for re-collection in babies <32/40 and/or <1500 g), without the need to increase the weight cut-off. Of concern, several clinically detected permanent and/or biochemically moderate CH cases (TSH $\geq$ 20 mIU/L) were associated with a TSH rise which occurred later than the final screening sample, but prior to NICU discharge. Although the increased

number of screen-detected cases cannot reliably be predicted, this suggests that screen detection of persistent or permanent CH would be improved by the addition of a final pre-discharge screening sample.

It is accepted that many preterm infants will have TSH blood concentrations within range on their initial screening test at 48–72 h of age, regardless of underlying congenital thyroid defects [15,22]. Due to the delayed TSH elevation in preterm infants, many programmes worldwide do additional screening in this group. There is no consensus on the optimal timing of repeat screening sample collection. Our New Zealand Low Birth Weight re-collection guideline was largely consistent with Australian programmes [2], but involves fewer re-collections than other countries (Table 3).

Table 3. Comparison of international newborn screening protocols.

State or Country	First Sample	Second Sample	Third Sample
Aotearoa New Zealand [17]	48–72 h	14 days if BW < 1500 g	28 days if BW < 1000 g
Queensland, Australia [2]	48–72 h	14 days if BW < 1500 g	28 days if BW < 1000 g
Western Australia, Australia [2]	48–72 h	14 days if BW < 1500 g	28 days if BW < 1000 g
South Australia, Australia [2]	At or near 48 h	10 days if BW < 1500 g	30 days or discharge if BW < 1500 g
Victoria, Australia [2,21]	36–72 h	4 weeks or discharge if BW < 1500 g or GA < 32 weeks	
New South Wales, Australia [2]	48–72 h	1 month if BW < 1500 g or GA < 30 weeks	
British Columbia, Canada [23]	24–48 h	21 days or day of discharge if BW < 1500 g	
United Kingdom [24]	5 days	28 days or day of discharge if GA < 32 weeks	
Wisconsin, USA [15]	24–48 h	14 days if BW < 2000 g or GA < 34 weeks	30 days for all infants
Japan [25]	5–7 days	4 weeks or body weight reaches 2500 g or at discharge if BW < 2000 g	

BW = birth weight, GA = gestational age at birth.

Vincent et al. [26] argued against re-screening in VLBW infants, as in their study of 465 infants screened initially at 2–5 days and again at 6 weeks of age, they only identified four cases of permanent primary congenital hypothyroidism, and all four were detected in the initial screening sample. Applying this strategy to the 13 cases in our audit, none of our cases were detected on the first screen, and it would have delayed diagnosis in seven infants who were detected on screens at 2 and 4 weeks of age. A study in Rhode Island [4] demonstrated that the delayed TSH elevation seen in preterm infants can be transient, with serum TSH normalising at a mean of 51 days of age. They hypothesised that re-screening may not be necessary; however, their neurodevelopmental follow-up found an increased incidence of infants with a head circumference <10th percentile at 18 months of age in the delayed TSH elevation group. Suboptimal head circumference measurements that persist beyond the initial neonatal period are associated with poorer neurodevelopmental outcomes [27]. In term infants, prompt normalisation of TSH and free T4 within the first two weeks of life is correlated with improved full-scale IQ [28]. Regardless of the permanence of disease as well as the difficulty in predicting transient disease, it would

seem justified to treat infants with a clinical picture of primary hypothyroidism. There are few long-term follow-up studies of preterm infants with delayed TSH elevation, and many of these infants receive levothyroxine supplementation, making long-term outcomes difficult to assess. Preterm infants as a population are already vulnerable to long-term disability and neurocognitive deficits [29], so it would seem prudent that all care should be taken to minimise further insults.

Current expert guidance from the international Clinical Laboratory Standards Institute (CLSI) is that a further newborn screening sample should be collected from all infants < 34/40 gestation or <2000 g birth weight, either at 28 days of age or discharge [30]. The European Society of Paediatric Endocrinology (ESPE) recommends that repeat specimen collection criteria be expanded further, i.e., that a repeat routine specimen be collected at 2 weeks postnatal age in all ill, preterm (<37 weeks gestational age), low-birth-weight infants (<2500 g) or multiple births, particularly same-sex twins, admitted to an NICU [9]. Since this audit was undertaken, the American Academy of Pediatrics (AAP) have updated guidance (in 2023) to recommend a repeat specimen at 2–4 weeks of age for infants < 32 weeks gestational age or <1500 g, and a further follow-up specimen if infant has not reached 36 weeks postmenstrual age at the time of the second sample [31].

New 'Preterm Metabolic Bloodspot Screening Protocol

The audit group undertook a stakeholder consultation process including neonatologists, regional paediatricians, paediatric endocrinologists, and screening laboratory scientists. The consensus was acceptance of the need to increase screening of preterm infants and that adding gestational age criteria in addition to weight-based criteria would not cause confusion or difficulty. The overwhelming preference was for a simple algorithm that was easy to follow. A new, simplified, and extended screening algorithm was developed (Figure 2), taking into account changes to sample timing and TSH thresholds that had occurred in the time since the audit occurred and analysis of results. This algorithm was presented to the Newborn Metabolic Screening Technical Working Group, which is responsible for technical oversight of the programme. The updated screening protocol was accepted in March 2024. The 'Preterm Metabolic Bloodspot Screening Protocol' replaced the previous 'Low Birth Weight Protocol' for infants with a birth weight under 1500 g from 1 July 2024.

The amendment of the protocol brings our practice in line with the recent AAP guideline recommending retesting of infants <1500 g and/or <32 weeks gestation [31] and incorporates the CLSI recommendation for follow-up at 28 days of age or discharge [30]. We did not find evidence to support the CLSI recommendation to broaden the inclusion criteria to <34/40 gestational age and birth weight of <2000 g. It is also not as broad as the European Society of Paediatric Endocrinology (ESPE) recommendation for further expansion of repeat specimen collection criteria, i.e., that a repeat routine specimen be collected at 2 weeks postnatal age in all ill, preterm (<37 weeks gestational age), low-birth-weight infants (<2500 g) or multiple births, particularly same-sex twins, admitted to an NICU [9]. Our protocol does not differentiate between well and unwell infants, and we no longer have different testing requirements for extremely low-birth-weight infants (<1000 g) in order to simplify the criteria for clinicians. Under the new preterm protocol, we estimate that an additional 1029 samples per year would be collected, including two or three repeat samples from 144 babies who are <32/40 but not <1500 g. This represents an increase of less than 2% of all national samples and a minimal additional cost. We intend to review this protocol in two years.

Neither proposed change (i.e., the addition of gestational age criteria and an additional pre-discharge sample) is anticipated to have a negative impact on screening for

disorders other than CH. Prematurity and illness both commonly lead to low T-Cell receptor excision circles (TREC) and high 17-hydroxy-progresterone (17-OHP) screening levels, and borderline-positive screen results for severe combined immunodeficiency (SCID) and congenital adrenal hyperplasia (CAH), respectively [32,33]. If the results do not normalise within the routine testing schedule, the screening programme may request a further blood-spot sample or recommend diagnostic testing, but the number of diagnostic referrals is kept as small as feasible. For CAH, the number of false-positive screens in sick and preterm babies has been dramatically reduced by reflexing samples with high 17-OHP levels to a highly specific second-tier test (steroid profile) [34]. For SCID, the protocol considers that a baby with a normal TREC result on any previous sample to have had a normal screen for SCID, even though subsequent TREC levels may drop below the threshold if the baby becomes more unwell. As such, the collection of additional samples may, in fact, (minimally) reduce the number of diagnostic referrals for SCID and CAH.

Figure 2. 'Preterm Metabolic Bloodspot Screening Protocol'; BW = birth weight. * Unless there has already been a bloodspot sample collected within 2 weeks.

Limitations of this audit are that it was restricted to infants within the Auckland region; however, it is Aotearoa New Zealand's largest city, comprising one-third of New Zealand's population, and includes two of the six neonatal intensive care units in the country. Our study was limited to infants under 34 weeks and 2000 g, so we were unable to comment on screening performance in ill, late preterm infants, or same-sex twins.

5. Conclusions

Findings that the low-birth-weight screening protocol was missing just under half of preterm and very low-birth-weight infants treated for primary CH prompted a stakeholder consultation and subsequent change in process. We have incorporated gestational age criteria and the addition of a pre-discharge sample to improve the sensitivity of the screening. This change is not anticipated to significantly increase the cost of screening or the workload of laboratory staff, but it provides a safeguard to minimise missed cases. Given their increased risk for long-term disabilities and neurocognitive challenges, all care

should be taken to prevent further harm to preterm infants. Our findings highlight a gap in preterm screening in Aotearoa New Zealand and possibly globally. Further modifications to preterm screening could include a lowering of the TSH thresholds to detect more cases; however, it appears that the timing of the sampling relative to the postnatal age of the infant may be more important. The delayed rise in TSH concentrations in preterm infants suggests the need for further expansion of preterm screening, and this area would benefit from a standardised approach. Further investigation into the aetiology of this marked increase in CH in this population is warranted.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Auckland Health Research Ethics Committee (AHREC) (AH2656, approved 2 July 2020) for studies involving humans. Locality approval was granted by Auckland, Counties Manukau, and Waitematā District Health Boards. The Newborn Screening Technical Working Group granted permission for analysis of screening data.

Informed Consent Statement: Participants in the New Zealand Newborn Metabolic Screening protocol consent to their data being used for programme monitoring and quality improvement.

Data Availability Statement: Data supporting the findings of the study are not publicly available in order to protect participant privacy and confidentiality. Participant consent for data access is restricted to screening programme monitoring and quality improvement. Interested parties may contact the authors for specific requests, subject to review and approval.

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Conflicts of Interest: Brown, Hofman and Heather are employees in Starship Child Health, New Zealand. Webster and Heather are employees in LabPlus, New Zealand. The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Ford, G.; LaFranchi, S.H. Screening for congenital hypothyroidism: A worldwide view of strategies. *Best Pract. Res. Clin. Endocrinol. Metab.* **2014**, *28*, 175–187. [CrossRef] [PubMed]
2. Huynh, T.; Greaves, R.; Mawad, N.; Greed, L.; Wotton, T.; Wiley, V.; Ranieri, E.; Rankin, W.; Ungerer, J.; Price, R.; et al. Fifty years of newborn screening for congenital hypothyroidism: Current status in Australasia and the case for harmonisation. *Clin. Chem. Lab. Med.* **2022**, *60*, 1551–1561. [CrossRef] [PubMed]
3. National Screening Unit. *Newborn Metabolic Screening Programme Annual Report 2021*; Ministry of Health: Wellington, New Zealand, 2023.
4. Woo, H.C.; Lizarda, A.; Tucker, R.; Mitchell, M.L.; Vohr, B.; Oh, W.; Phornphutkul, C. Congenital hypothyroidism with a delayed thyroid-stimulating hormone elevation in very premature infants: Incidence and growth and developmental outcomes. *J. Pediatr.* **2011**, *158*, 538–542. [CrossRef] [PubMed]
5. Bijarnia, S.; Wilcken, B.; Wiley, V.C. Newborn screening for congenital hypothyroidism in very-low-birth-weight babies: The need for a second test. *J. Inherit. Metab. Dis.* **2011**, *34*, 827–833. [CrossRef]

6. Albert, B.B.; Cutfield, W.S.; Webster, D.; Carll, J.; Derraik, J.G.B.; Jefferies, C.; Gunn, A.J.; Hofman, P.L. Etiology of increasing incidence of congenital hypothyroidism in New Zealand from 1993–2010. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 3155–3160. [CrossRef]
7. McGrath, N.; Hawkes, C.P.; Mayne, P.; Murphy, N.P. Optimal Timing of Repeat Newborn Screening for Congenital Hypothyroidism in Preterm Infants to Detect Delayed Thyroid-Stimulating Hormone Elevation. *J. Pediatr.* **2019**, *205*, 77–82. [CrossRef]
8. LaFranchi, S.H. Thyroid Function in Preterm/Low Birth Weight Infants: Impact on Diagnosis and Management of Thyroid Dysfunction. *Front. Endocrinol.* **2021**, *12*, 666207. [CrossRef]
9. Léger, J.; Olivieri, A.; Donaldson, M.; Torresani, T.; Krude, H.; van Vliet, G.; Polak, M.; Butler, G.; ESPE-PES-SLEP-JSPE-APEG-APPES-ISPAE; Congenital Hypothyroidism Consensus Conference Group. European Society for Paediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. *Horm. Res. Paediatr.* **2014**, *81*, 80–103. [CrossRef]
10. Raymond, J.; LaFranchi, S.H. Fetal and neonatal thyroid function: Review and summary of significant new findings. *Curr. Opin. Endocrinol. Diabetes Obes.* **2010**, *17*, 1–7. [CrossRef]
11. Ares, S.; Saenz-Rico, B.; Arnaez, J.; Diez-Sebastian, J.; Omeñaca, F.; Bernal, J. Effects of oral iodine supplementation in very low birth weight preterm infants for the prevention of thyroid function alterations during the neonatal period: Results of a randomised assessor-blinded pilot trial and neurodevelopmental outcomes at 24 months. *Eur. J. Pediatr.* **2022**, *181*, 959–972. [CrossRef]
12. New Zealand Ministry of Health. Report on Maternity Web Tool. Available online: https://tewhatuora.shinyapps.io/report-on-maternity-web-tool/ (accessed on 1 June 2023).
13. Chow, S.S.W.; Creighton, P.; Chambers, G.M.; Lui, K. *Report of the Australian and New Zealand Neonatal Network 2020*; ANZNN: Sydney, Australia, 2022.
14. Statistics New Zealand. 2018 Census Place Summaries. Available online: https://www.stats.govt.nz/tools/2018-census-place-summaries/new-zealand (accessed on 25 June 2024).
15. Kaluarachchi, D.C.; Allen, D.B.; Eickhoff, J.C.; Dawe, S.J.; Baker, M.W. Increased Congenital Hypothyroidism Detection in Preterm Infants with Serial Newborn Screening. *J. Pediatr.* **2019**, *207*, 220–225. [CrossRef] [PubMed]
16. Odenwald, B.; Fischer, A.; Röschinger, W.; Liebl, B. Long-term course of hypothyroidism detected through neonatal TSH screening in a population-based cohort of very preterm infants born at less than 32 weeks of gestation. *Int. J. Neonatal Screen.* **2021**, *7*, 65. [CrossRef] [PubMed]
17. National Screening Unit. *Guidelines for Practitioners Providing Services Within the Newborn Metabolic Screening Programme in New Zealand*; National Screening Unit: Wellington, New Zealand, 2010.
18. CLSI. *Newborn Screening for Congenital Hypothyroidism*; First Edition Clinical and Laboratory Standards Institute: Berwyn, PA, USA, 2024.
19. Statistics New Zealand. *Live Births by Area, Regional Councils (Maori and Total Population) (Annual-Dec)*; Statistics New Zealand: Wellington, New Zealand, 2019.
20. Kaluarachchi, D.C.; Allen, D.B.; Eickhoff, J.C.; Dawe, S.J.; Baker, M.W. Thyroid-Stimulating Hormone Reference Ranges for Preterm Infants. *Pediatrics* **2019**, *144*, e20190290. [CrossRef]
21. Greaves, R.F.; Northfield, J.-A.; Cross, L.; Mawad, N.; Nguyen, T.; Tan, M.; O'Connell, M.A.; Pitt, J. Managing newborn screening repeat collections for sick and preterm neonates. *Int. J. Neonatal Screen.* **2024**, *10*, 63. [CrossRef]
22. Vigone, M.C.; Caiulo, S.; Di Frenna, M.; Ghirardello, S.; Corbetta, C.; Mosca, F.; Weber, G. Evolution of thyroid function in preterm infants detected by screening for congenital hypothyroidism. *J. Pediatr.* **2014**, *164*, 1296–1302. [CrossRef]
23. The Provincial Health Services Authority (PHSA). *Neonatal Guideline: Newborn Metabolic Screening*; Perinatal Services BC: Vancouver, BC, Canada, 2024.
24. Public Health England. PHE Screening. In *Guidelines for Newborn Blood Spot Sampling*; Public Health England: London, UK, 2016.
25. Minamitani, K. Newborn screening for congenital hypothyroidism in Japan. *Int. J. Neonatal Screen.* **2021**, *7*, 34. [CrossRef]
26. Vincent, M.A.; Rodd, C.; Dussault, J.H.; Van Vliet, G. Very low birth weight newborns do not need repeat screening for congenital hypothyroidism. *J. Pediatr.* **2002**, *140*, 311–314. [CrossRef]
27. Hayes, B.; Lee, K. Head size and growth in the very preterm infant: A literature review. *Res. Rep. Neonatol.* **2015**, *5*, 1–7. [CrossRef]
28. Selva, K.A.; Harper, A.; Downs, A.; Blasco, P.A.; Lafranchi, S.H. Neurodevelopmental outcomes in congenital hypothyroidism: Comparison of initial T4 dose and time to reach target T4 and TSH. *J. Pediatr.* **2005**, *147*, 775–780. [CrossRef]
29. Joseph, R.M.; O'Shea, T.M.; Allred, E.N.; Heeren, T.; Hirtz, D.; Jara, H.; Leviton, A.; Kuban, K.C.K.; ELGAN Study Investigators. Neurocognitive and Academic Outcomes at Age 10 Years of Extremely Preterm Newborns. *Pediatrics* **2016**, *137*, e20154343. [CrossRef]
30. CLSI. *Newborn Screening for Preterm, Low Birth Weight, and Sick Newborns*; Approved Guideline CLSI Document NBS03-A; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2009.

31. Rose, S.R.; Wassner, A.J.; Wintergerst, K.A.; Yayah-Jones, N.-H.; Hopkin, R.J.; Chuang, J.; Smith, J.R.; Abell, K.; LaFranchi, S.H.; Section on Endocrinology Executive Committee; et al. Congenital Hypothyroidism: Screening and Management. *Pediatrics* **2023**, *151*, e2022060420. [CrossRef] [PubMed]
32. Heather, N.; de Hora, M.; Brothers, S.; Grainger, P.; Knoll, D.; Webster, D. Introducing Newborn Screening for Severe Combined Immunodeficiency—The New Zealand Experience. *Screening* **2022**, *8*, 33. [CrossRef] [PubMed]
33. de Hora, M.R.; Heather, N.L.; Webster, D.R.; Albert, B.B.; Hofman, P.L. Evaluation of a New Laboratory Protocol for Newborn Screening for Congenital Adrenal Hyperplasia in New Zealand. *Screening* **2022**, *8*, 56. [CrossRef] [PubMed]
34. de Hora, M.R.; Heather, N.L.; Patel, T.; Bresnahan, L.G.; Webster, D.; Hofman, P.L. Implementing steroid profiling by liquid chromatography-tandem mass spectrometry improves newborn screening for congenital adrenal hyperplasia in New Zealand. *Clin. Endocrinol.* **2021**, *94*, 904–912. [CrossRef]

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Newborn Genetic Screening Improves the Screening Efficiency for Congenital Hypothyroidism: A Prospective Multicenter Study in China

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Abstract: Newborn congenital hypothyroidism (CH) screening has been widely used worldwide. The objective of this study was to evaluate the effectiveness of applying biochemical and gene panel sequencing as screening tests for CH and to analyze the mutation spectrum of CH in China. Newborns were prospectively recruited from eight hospitals in China between February and December 2021. Clinical characteristics were collected. Second-generation sequencing was used to detect four CH-related genes, and the genetic patterns of the pathogenic genes were analyzed. We analyzed the relationship between genotype and biochemical phenotype. A total of 29,601 newborns were screened for CH. Gene panel sequencing identified 18 patients, including 10 patients affected by biochemically and genetically screened disorders and 8 patients affected by solely genetically screened disorders. The predictive positive value of genetic screening was 34.62%, which was much greater than that of biochemical screening alone (17.99%). A total of 94 cases of congenital thyroid dysfunction were confirmed by biochemical and genetic screening, including 30 CHs and 64 isolated hyperthyrotropinemia (HTT), with an incidence of 1/987 for CH and 1/463 for HTT, and a total incidence of 1/315 for hypothyroidism. The incidence rate and number of patients in Jinan were the highest, and the incidence rates in Shijiazhuang and Shanghai were the lowest. The gene mutation rate in this study was 19.1%, mainly *DUOX2* mutation. The most common variant of *DUOX2* was c.1588A>T(p.Lys530*). There was only a difference in sFT4 between groups with gene mutations and those without mutations. Genetic screening is a supplement to biochemical screening. Combining biochemical screening with genetic screening is useful for improving screening efficiency. The incidence of CH in China according to a multicenter study of nearly 30,000 NBS surveys was 1/315. *DUOX2* gene mutations are commonly detected in these patients.

Keywords: congenital hypothyroidism; newborn screening; genetic screening; *DUOX2*

1. Introduction

Congenital hypothyroidism (CH) is one of the most common neonatal endocrine and metabolic diseases and affects approximately 1:2000–1:4000 infants worldwide [1]. According to newborn screening data, the incidence rate of CH in China is higher than the global average level [2,3]. CH can lead to growth retardation and permanent intellectual disability [4]. However, early screening and treatment could significantly improve adverse outcomes. Thus, most countries conduct newborn screening (NBS) for CH, which is widely used as the third preventive intervention for birth defects [5,6].

Traditional biochemical screening for thyroid stimulating hormone (TSH) from the dried blood spot (DBS) is used as the mainstream method of CH screening at this stage [7]. However, TSH levels are affected by many factors, such as gestational age, birth weight, feeding, and basic diseases [8–10]. With increasing research on the pathogenesis of CH, the genetic origin of its pathogenesis has gradually been recognized [11,12]. According to the latest report, newborn genetic screening has been proven to be successful when single monogenetic disease or targeted genetic sequencing panels are used [13,14]. However, no study has evaluated the effectiveness of combined biochemical screening and genetic screening for CH in a large population. Moreover, the incidence of CH NBS in the Chinese population has been reported, but there is a lack of joint screening studies for multiple genes and related research on CH-related genes and clinical phenotypes [15].

This study employed targeted genetic sequencing to detect four candidate genes related to CH and combined it with biochemical screening to determine the latest incidence of CH in China. Furthermore, the study analysed the relationship between the genotype and clinical phenotype of children with CH and the mutation spectrum of CH pathogenic genes in selected Chinese populations. In addition, the study laid a theoretical foundation for neonatal screening and clinical diagnosis of CH and future gene therapy.

2. Materials and Methods

2.1. Study Population and Design

From 21 February 2021 to 30 December 2021, a total of 29,601 newborn infants who participated in the newborn screening program and gene screening project were recruited for this study. Eight hospitals participated in the multicenter project, including all the subjects who received CH screening via DBS collection. Each participating unit should include appropriate subjects. Leaflets of the free gene screening program for newborns should be distributed among the guardians of the subjects. They should be informed and should sign an informed consent to confirm the inclusion of the subjects. The study design and protocol were reviewed and approved by the Ethics Committee of Xinhua Hospital Affiliated with Shanghai Jiaotong University School of Medicine. The eight hospitals were selected to represent the nationwide population, as they are regional tertiary hospitals located in East China, West China, South China, and North China. The participating hospitals were Xinhua Hospital affiliated with Shanghai Jiaotong University School of Medicine, Guangzhou Women and Children's Medical Center, Jinan Maternity and Child Care Hospital, Shijiazhuang Maternal and Child Health Care Hospital, Chongqing Health Center for Women and Children, the First People's Hospital of Yunnan Province, Inner Mongolia Maternity and Child Health Care Hospital, and Hainan Women and Children's Medical Center.

2.2. Newborn Screening

The screening, diagnosis, treatment, and reevaluation of CH were in line with the criteria of the "Consensus statement on the diagnosis and management of congenital hypothyroidism" in China [16]. Briefly, for 7 days after 72 h from birth, the newborns

were exclusively breastfed, and blood was collected from the heel and dripped on special filter paper (Whatman903, Liding, Guizhou, China) to form DBSs. A time-resolved fluoroimmunoassay (Perkin-Elmer, Waltham, MA, USA) was used to measure the TSH level. The cutoff value of TSH varies according to the different screening methods used at each hospital (see Supplementary Table S1 for specific values). If the TSH level elevated the upper limit of normal, the infants would be recalled, heel blood would be collected for a second time and the TSH level would be retested. If the new TSH value was still above the upper limit of normal, the infants would be recalled again, and their venous blood would be collected to measure the levels of serum TSH and FT4.

2.3. Diagnosis of CH

TSH Reagent Kit (Abbott, IL, USA) and Free T4 Reagent Kit (Abbott, IL, USA) were used to measure Serum TSH and FT4 levels. Serum TSH and FT4 levels were determined via an electrochemiluminescence immunoassay (ECLIA) assay, ARCHITECT i system, and Alinity i analysis System (Abbott, IL, USA). The diagnosis of CH is based on elevated TSH levels and decreased FT4 levels. Isolated hyperthyrotropinemia (HTT) is characterized by increased TSH and normal FT4. The cutoff values of serum TSH and FT4 vary according to the different screening methods used at each hospital (Supplementary Table S1 for specific values). Thyroid ultrasonography was performed to evaluate thyroid development. The information regarding the diagnosed children, including birth time, sex, birth weight, gestational week, and family history of thyroid disease, was collected and recorded via the neonatal disease screening registration form. The screening procedure is shown in Figure 1.

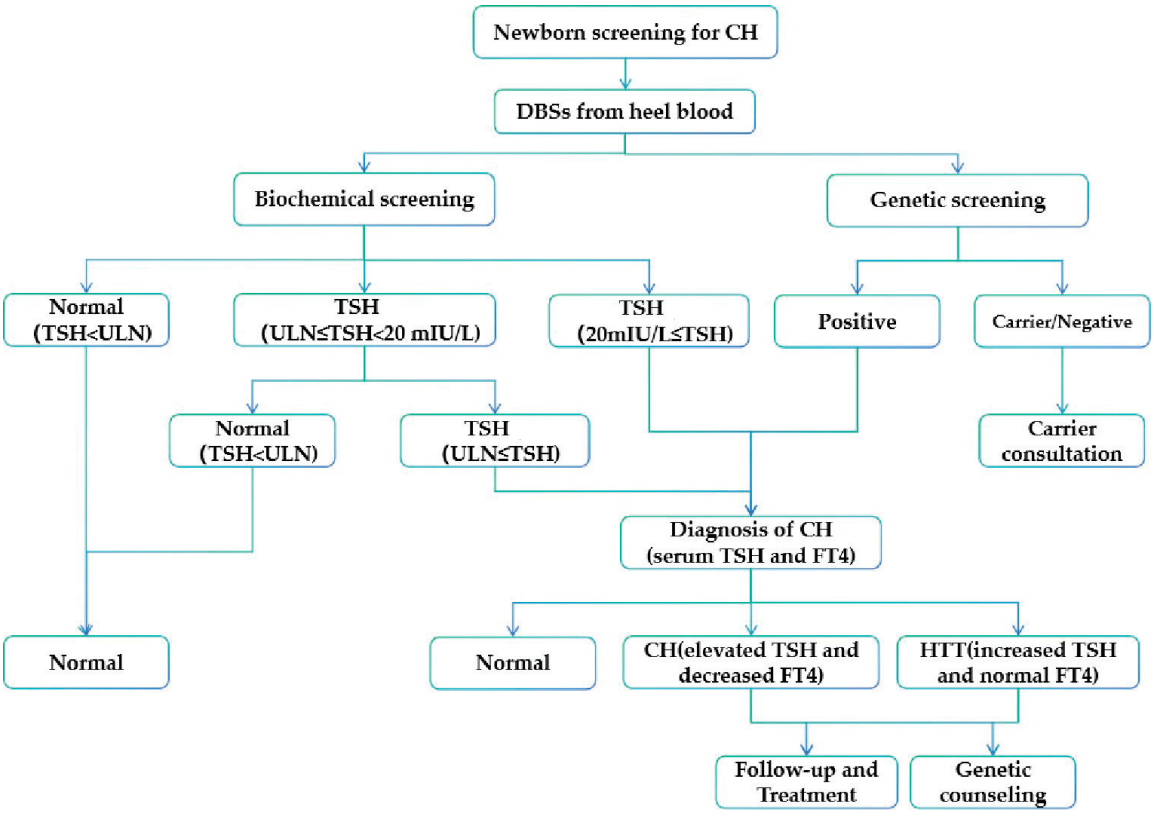

Figure 1. The screening and diagnosis process of CH. CH, congenital hypothyroidism; HTT, isolated hyperthyrotropinemia; ULN, upper limit of normal value; TSH, thyroid stimulating hormone.

2.4. Genomic DNA Extraction and Sequencing

Genomic DNA was extracted from the DBS using a genomic DNA extraction system kit (QIAamp DNA Blood Midi Kit, Qiagen, Hilden, Germany). The DNA concentration was 3–25 ng/μL, and the DNA purity (OD 260/280) reached 1.8–2.0. The genomic DNA

was broken into small DNA fragments with a main band of 100–500 bp by a Covaris LE220 ultrasonic instrument (MA, USA), and then the broken DNA fragments were screened by magnetic beads. Library construction, end repair, and 3′-end tailing were performed using a fragmentation module (Vazyme, Nanjing, China). The four CH-related genes *DUOX2* (NM 014080), *DUOXA2* (NM 207581), *TSHR* (NM 000369), and *PROP1* (NM 006261) were selected via a gene capture strategy via the Agilent 2100 Bioanalyzer (Beijing, China) and BMG following the manufacturer's protocol. High-throughput sequencing of the qualified enriched libraries was performed on a MEGISEQ-2000 sequencer (BGI, Shenzhen, China).

2.5. Analysis of Mutation Data

The sequencing results were analyzed via bioinformatics methods. Spilt, comparison, and quality control were performed on the original sequencing data. We performed a search of internal databases, dbSNP, ESP6500, gnomAD, and 1000 Genomes. Prediction software was then used to predict whether the mutations were conserved and the contribution of the mutations to CH pathogenesis. We searched the HGMD, PubMed, Clinvar, and other databases and literature related to variation, and variants were analyzed following the basic criteria from the American College of Medical Genetics (ACMG) guidelines [17,18].

2.6. Treatment and Follow-Up

After diagnosis, all the children were given L-T4 (levothyroxine sodium) therapy, their thyroid function was assessed after one month of treatment, and the dosage was adjusted according to the test results of thyroid function, height, weight, and individual differences. Under the condition of normal thyroid function, the patient was rechecked at 2–3 months of age and once every 3–4 months of age from 1 to 3 years of age, and physical and intellectual development was evaluated regularly.

2.7. Statistical Analysis

Statistical analysis was performed via SPSS (version 25.0, NY, IBM Corp.). The chi-squared test was used to compare counting data between groups. A test of normal distribution and homogeneity of variance was used for measurement data. Data that were not normally distributed are expressed as medians (M), 25th percentiles (P25), and 75th percentiles (P75). Normally distributed data are expressed as the means and standard deviations. The difference between each group was tested by a non-parametric rank sum test. $p < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Newborn Screening for CH

In this study, a total of 29,601 newborns underwent CH screening. A total of 94 cases of congenital thyroid dysfunction were confirmed, among which 46 were male infants and 48 were female infants. The incidence rate of hypothyroidism was 1/315. According to the clinical phenotype, there were 30 CHs and 64 HTTs in 94 patients. The gestational ages were mostly between 37 and 42 weeks, and only four infants (4%) were preterm. Birth weights were normal in 89 cases, low in 2 infants (2%), and macrosomic in 3 newborns (3%). The median blood spot TSH levels of the 94 patients was 11.52 mIU/L (P25–P75: 9.03–16.93 mIU/L). After serological examination, the median serum TSH level of CH infants was 20.28 μIU/mL (P25–P75: 12.11–49.80 μIU/mL), and that of FT4 was 13.65 pmol/L (P25–P75: 9.55–15.38 pmol/L). Among them, the incidence rate and number of patients in Jinan were the highest, followed by those in Chongqing and Yunnan, and the incidence rates in Shijiazhuang and Shanghai were the lowest (Tables 1 and 2).

Table 1. Multicenter screening for congenital hypothyroidism.

Region	Screening Number	CH	HTT	Total	Incidence Rate
Shanghai	4888	1	3	4	1/1222
Jinan	4797	7	40	47	1/102
Guangzhou	4813	7	3	10	1/481
Hainan	977	1	1	2	1/489
Chongqing	2988	10	7	17	1/176
Yunnan	3006	3	8	11	1/273
Shijiazhuang	4899	1	0	1	1/4899
Inner Mongolia	3233	0	2	2	1/1616
Total	29,601	30	64	94	1/315

Table 2. Clinical features of the 94 cases.

Project	Cases (%)	Project	Cases (%)
Gender		Clinical phenotype	
Male	46 (49%)	CH	30 (32%)
Female	48 (51%)	HTT	64 (68%)
Gestational age (week)		blood spot TSH (mIU/L)	
<37	4 (4%)	<10	35 (37%)
37–42	90 (96%)	10–40	48 (51%)
≥42	0	≥40	11 (12%)
Birth weight (g)		Serum TSH (μIU/mL)	
<2500	2 (2%)	5–20	47 (50%)
2500–4000	89 (95%)	20–100	33 (35%)
≥4000	3 (3%)	≥100	14 (15%)
Gene mutation		Serum FT4 (pmol/mL)	
NO variants	76 (81%)	<6	7 (7%)
DUOX2 variants	18 (19%)	6–12	33 (35%)
		≥12	54 (58%)

3.2. Comparison of Biochemical Screening and Genetic Screening Results

In total, 499 infants had positive traditional biochemical results, and the positive rate of initial screening was 1.68% (499/29,601). A total of 478 (95.79%) cases were successfully recalled and underwent serological confirmation testing. According to the serological confirmation test, 86 infants (43 males and 43 females) were diagnosed with hypothyroidism. The positive predictive value of biochemical screening was 17.99% (86/478). The recorded demographic data and clinical features of the patients are presented in Supplementary Tables S2 and S3. CH-related genes were detected by targeted next-generation sequencing (NGS) in 29,601 newborns. In total, 62 strains tested positive via genetic screening, and the positive rate of genetic screening was 0.21% (62/29,601). A total of 59 patients (95.2%) were successfully recalled and underwent a serological confirmation test. According to the serological confirmation test, 18 infants were diagnosed with hypothyroidism, including 6 with CH and 12 with HTT. The positive predictive value of genetic screening was 34.62% (18/59), which was greater than that of biochemical screening. The above results suggest that genetic screening improves the detection rate of hypothyroidism.

We then compared positive results from traditional biochemical screening and genetic screening. According to the results of the combined genetic screening and traditional biochemical screening, 94 children were diagnosed. Among them, 76 were positive in biochemical screening and negative in genetic screening, 10 were positive in both types of screening, and 8 were negative in biochemical screening and positive in genetic screening. The above results indicate that 8 out of every 94 patients were missed by traditional biochemical screening; fortunately, they could be identified by genetic screening. Genetic screening plays an important role in improving the efficiency of CH screening and could be used as a supplement to biochemical screening. Compared with those of combined screening, the sensitivities of traditional biochemical screening and genetic screening were

91.49% and 19.15%, respectively. Combined screening improves the sensitivity of screening (Table 3).

Table 3. Diagnostic results.

		Combined Screening		Predictive Value	Total
		+	−		
Biochemical screening	+	86	0	PPV: 100%	86
	−	8	29,507	NPV: 99.97%	29,515
		Sensitivity: 91.4%	Specificity: 100%		
Genetic screening	+	18	0	PPV: 100%	18
	−	76	29,507	NPV: 99.74%	29,583
		Sensitivity: 19.14%	Specificity: 100%		
Total		94	29,504		29,601

+, positive screening; −, negative screening; PPV, Positive Predictive Value; NPV, Negative Predicticv Value.

3.3. Mutation Patterns of CH-Related Genes

On the basis of our literature review, we designed a targeted sequencing panel that included four causative genes: *DUOX2*, *DUOXA2*, *TSHR*, and *PROP1*. Because of the critical role of the above four genes in thyroid hormone synthesis and action, and the strong correlation between their mutations and CH, they were included in the gene sequencing panel for CH. Among the 29,601 newborns, 62 tested positive for the *DUOX2* gene, which is related to thyroid dyshormonogenesis. Eighteen patients with pathogenic variants in the *DUOX2* gene were eventually diagnosed with hypothyroidism. As shown in Table 4, the most common variant of *DUOX2* was c.1588A>T(p.Lys530*) with a frequency of 33.33%, followed by c.3329G>A(p.Arg1110Gln) (11.11%). Among the 16 mutation sites of *DUOX2*, 6 were located in the peroxidase-like domain, 4 in the ferric oxidoreductase domain, 3 in the EF-hand domain, and 1 in the FAD-binding FR-type domain, all of which play key roles in the function of *DUOX2*. Only the c.1883delA(p.Lys628Argfs*) and c.2048G>T(p.Arg683Leu) were located in the nonfunctional domain (Figure 2). No mutations in *TSHR*, *DUOXA2*, or *PROP1* were detected.

Table 4. Potential pathological variants detected in the present study.

Gene	Position	cDNA Change	Amino Acids Change	ACMG Classification	Mutation Type	No. of Cases	Frequency (%)
DUOX2	Exon14	c.1588A>T	p.Lys530*	P	nonsense	12	33.33%
DUOX2	Exon25	c.3329G>A	p.Arg1110Gln	P	missense	4	11.11%
DUOX2	Exon10	c.2635G>A	p.Glu879Lys	P	missense	3	8.33%
DUOX2	Exon20	c.2654G>T	p.Arg885Leu	LP	missense	3	8.33%
DUOX2	Exon16	c.1883delA	p.Lys628Argfs*	P	frameshift	2	5.55%
DUOX2	IVS28	c.3693+1G>T	/	LP	splicing	2	5.55%
DUOX2	Exon5	c.477delC	p.Glu160Argfs*	LP	frameshift	1	2.78%
DUOX2	Exon6	c.596delC	p.Ser199Trpfs*	LP	frameshift	1	2.78%
DUOX2	Exon6	c.605_621delAGCTGGCGTCGGGGCCC	p.Gln202fs*	LP	frameshift	1	2.78%
DUOX2	Exon9	c.978_979delGGinsTT	p.Glu327*	LP	frameshift	1	2.78%
DUOX2	Exon15	c.1708C>T	p.Gln570*	LP	nonsense	1	2.78%
DUOX2	Exon17	c.2048G>T	p.Arg683Leu	LP	missense	1	2.78%
DUOX2	Exon20	c.2654G>A	p.Arg885Gln	P	missense	1	2.78%
DUOX2	Exon25	c.3285_3286delTT	p.Ile1097Leufs*	LP	frameshift	1	2.78%
DUOX2	Exon27	c.3516_3531delGTCCAAGCTTCCCCAG	p.Lys1174Serfs*	P	frameshift	1	2.78%
DUOX2	Exon30	c.4000C>T	p.Arg1334Trp	LP	missense	1	2.78%
Total						36	100%

P, pathogenic; LP, likely pathogenic.

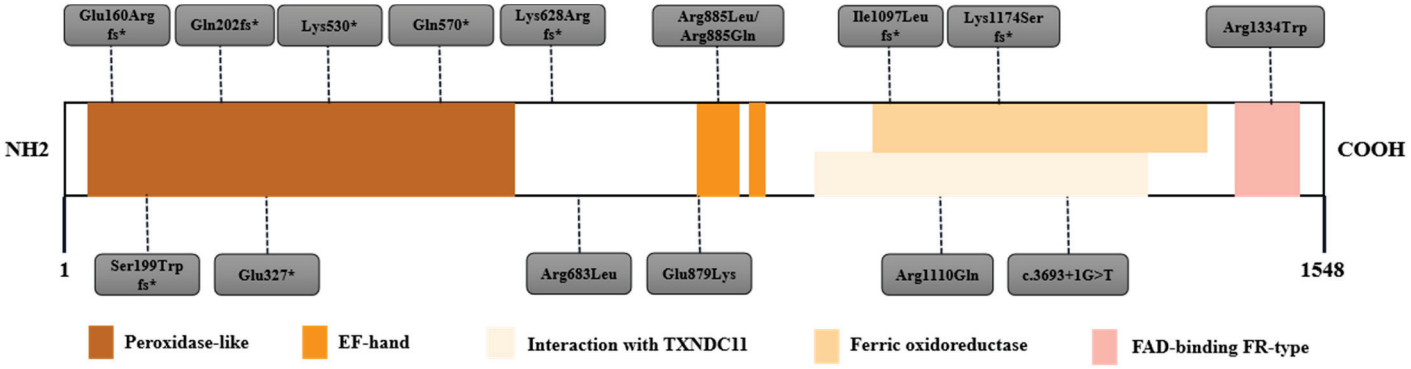

Figure 2. Mutation sites in the secondary structure of *DUOX2*. Sixteen mutation sites distributed in the secondary structure of *DUOX2* protein. *DUOX2* contains 5 functional domains, peroxidase-like domain, EF-hand domain, Interaction with TXNDC11 domain, ferric oxidoreductase domain, and FAD-binding FR-type domain.

3.4. Relationships Between Genotype and Phenotype

Ninety-four children were divided into two groups, CH and HTT, on the basis of their clinical phenotype. The biochemical indices of thyroid function were compared between the two groups (Figure 3). The results revealed that the dry blood spots and serum TSH levels of patients with CH were significantly greater than those of patients with HTT ($p < 0.0001$). The median sTSH concentration at diagnosis was greater in patients with CH than in those with HTT ($p < 0.0001$). The median FT4 concentration at diagnosis was greater in patients with HTT than in those with CH ($p < 0.0001$).

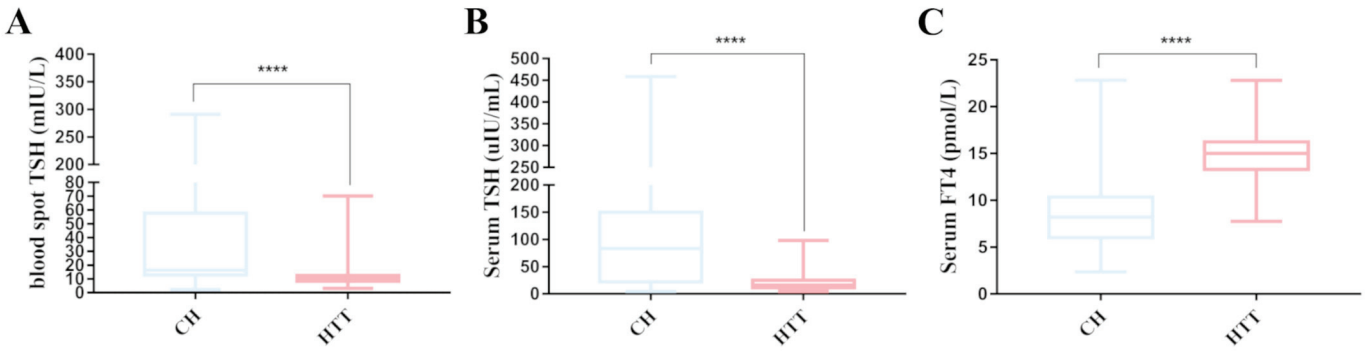

Figure 3. Comparison of serum levels of screening TSH, diagnostic TSH and FT4 among different groups, classified according to clinical phenotype. (**A**) Comparison of TSH levels in dry blood spots between patients with CH and HTT. (**B**) Comparison of TSH levels in serum between patients with CH and HTT. (**C**) Comparison of FT4 levels in serum between patients with CH and HTT. CH, Congenital hypothyroidism; HTT, isolated hyperthyrotropinemia; **** $p < 0.0001$.

According to the results of genetic screening, the studied patients were classified into two groups, and the biochemical indices of thyroid function were compared between the two groups (mutation group and no mutation group). The results revealed that only the serum FT4 levels of patients with mutations at diagnosis were significantly lower than those of patients without gene mutations ($p < 0.01$) (Figure 4). There was no significant difference in the levels of blood spot TSH and sTSH between the mutation and non-mutation groups.

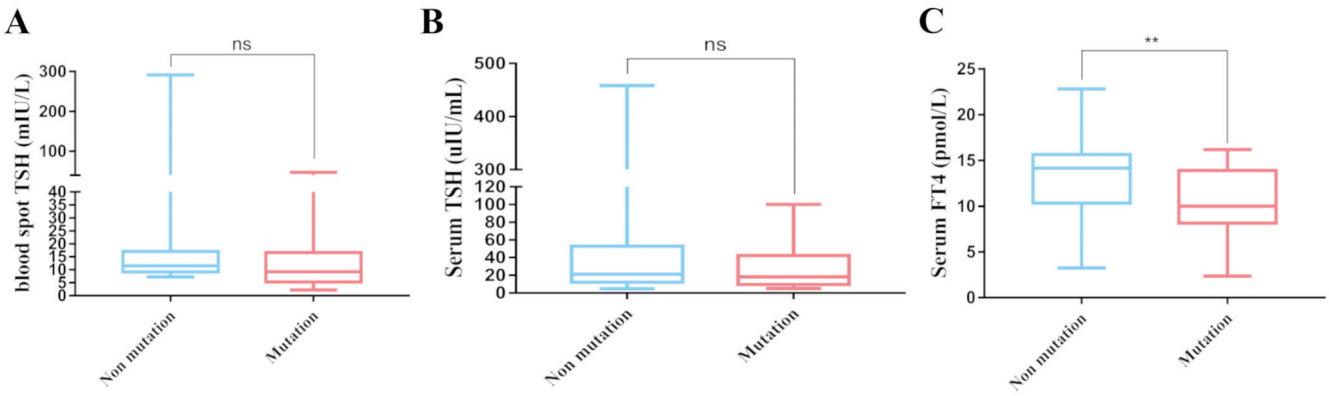

Figure 4. Comparison of serum levels of screening TSH, diagnostic TSH and FT4 among different groups, classified according to whether there is a gene variation. (**A**) Comparison of TSH levels in dry blood spots between patients with no mutation, and mutation. (**B**) Comparison of TSH levels in serum between patients with no mutation, and mutation. (**C**) Comparison of FT4 levels in serum between patients with no mutation, and mutation. ns, $p > 0.05$; ** $p < 0.01$.

Next, we investigated the relationship between the genotype and clinical phenotype of CH patients. Because many types of gene mutations are detected, the c.1588A>T and c.3329G>A mutations and the mutation types with the highest mutation frequency are taken as genotypes. We studied the relationship between the genotypes of high-frequency gene mutation sites and the clinical phenotypes of CH patients (Tables 5 and 6) and found that the c.1588A>T and c.3329G>A variants were not related to clinical type (CH or HTT). In addition, significant differences in sFT4 levels were detected between patients with no c.1588A>T variant, the c.1588A>T homozygous variant, and the c.1588A>T compound heterozygous variant, indicating that patients with the c.1588A>T variant are associated with low sFT4 levels.

Table 5. Relationship between the genotype of c.1588A>T variant and the biochemical results of thyroid function.

Groups	Cases	CH (*n*)	HTT (*n*)	Blood Spot TSH (mIU/L)	Serum TSH (uIU/mL)	Serum FT4 (pmol/L)
No c.1588A>T variant	85	26	59	11.29 (9.01, 16.72)	20.99 (11.68, 45.02)	14.05 (10.27, 16.29)
c.1588A>T homozygous variant	3	2	1	13.84 (12.47, -)	18.41 (17.07, -)	9.62 (5.62, -)
c.1588A>T compound heterozygous variant	6	4	2	16.38 (5.66, 31.14)	24.12 (11.67, 72.64)	9.07 (6.42, 11.50)
Statistics		$X^2 = 4.718$; $p = 0.095$		H = 0.759; p = 0.684	H = 0.278; p = 0.870	H = 8.592; p = 0.014 *

CH, Congenital hypothyroidism; HTT, isolated hyperthyrotropinemia. * $p < 0.05$.

Table 6. Relationship between the genotype of c.3329G>A variant and the biochemical results of thyroid function.

Groups	Cases	CH (*n*)	HTT (*n*)	Blood Spot TSH (mIU/L)	Serum TSH (uIU/mL)	Serum FT4 (pmol/L)
No c.3329G>A variant	91	29	62	11.60 (9.03, 16.77)	20.99 (12.23, 54.28)	13.65 (9.35, 15.43)
c.3329G>A homozygous variant	1	0	1	9.30 (9.30, 9.30)	17.92 (17.92, 17.92)	12.79 (12.79, 12.79)
c.3329G>A compound heterozygous variant	2	1	1	7.70 (6.32, -)	21.27 (9.88, -)	11.17 (10.72, -)

Table 6. *Cont.*

Groups	Cases	CH (*n*)	HTT (*n*)	Blood Spot TSH (mIU/L)	Serum TSH (uIU/mL)	Serum FT4 (pmol/L)
Statistics		$X^2 = 0.770$; $p = 0.680$		H = 3.003; p = 0.223	H = 0.215.; p = 0.898	H = 0.947; p = 0.623

CH, Congenital hypothyroidism; HTT, isolated hyperthyrotropinemia.

4. Discussion

CH is an endocrine disease caused by insufficient synthesis and secretion of thyroid hormones. It is one of the major targets of newborn screening in the world. This study presented nearly 30,000 NBS data points, indicating that the average incidence rate of hypothyroidism (including CH and HTT) in multiple regions was 1/315, which is far higher than the reported incidence rate [1–3]. Among them, the incidence rate of CH was 1/897, and the incidence rate of HTT was 1/467. Studies have suggested that the increasing trend of CH incidence might be associated with a reduction in TSH cutoff levels, an increase in the survival of preterm infants, and changes in population demographics [19–21]. An increased proportion of CH patients with eutopic glands may also account for this trend [2]. Furthermore, the variability of serum reference ranges (RRs) and the absence of specific neonatal RRs may contribute to an increased likelihood of CH or HTT being misdiagnosed. The question of whether infants with normally positioned glands and mild HTT should receive treatment remains unresolved. Some studies have indicated that it is safe to not treat infants with mild HTT without significant developmental deficits, while others have supported early treatment as a means of preventing potential long-term cognitive problems. Consequently, a follow-up plan must be tailored to the specific circumstances of the child. It is also essential to have a clear understanding of how to manage the dose and course of LT4 [22,23].

Our data revealed that the incidence rate and the number of patients in Jinan were the highest, followed by those in Chongqing and Yunnan and that the incidence rates in Shijiazhuang and Shanghai were the lowest. This finding is consistent with the regional differences in the incidence of CH [21,24]. This may be related to the iodine distribution in the area and the residents' eating habits. Additionally, differences in CH incidence across geographic regions may also be related to the distribution of populations with CH susceptibility genes. The genetic pathogenesis of CH in most newborns is still unclear, and some cases are related to genetic mutations [25], which are often undetectable by NBS.

NBS for CH is performed routinely in most regions of China, where it has led to the near elimination of intellectual disability caused by this common condition. However, NBS for CH is associated with a high rate of false-negative results, which appears to be inevitable because the TSH and FT4 concentrations at birth are easily affected by maternal and other factors. These factors include premature birth, low birth weight, and central hypothyroidism [9,10]. Furthermore, false positives can cause newborns and their families to be recalled to the hospital for reexamination, which may take a long time. Additionally, newborns at risk of CH may not produce enough TSH in the first few weeks after birth, and the screening results may be false negative at this time. The guidelines recommend that for twins, a second screening and follow-up should be performed 2 weeks after birth or 2 weeks after the first screening. However, it is important to note that not all subsequent cases of hypothyroidism are cases of CH that have been overlooked. It is therefore essential to perform a thorough differential diagnosis. In our study, genetic screening was performed on 29,601 neonates via targeted next-generation sequencing, and 8 false-negative CH cases were identified, which translates into 8 out of every 94 newborns benefiting from the implementation of CH genetic screening. Following the incorporation of the aforementioned eight children into the follow-up treatment system, it was observed that they exhibited normal thyroid development, thyroid function, neuropsychiatric development, and growth and development. The above results and those of previous studies confirmed

the effectiveness of genetic screening, which could be used as a supplement to biochemical screening [26,27]. Therefore, NBS for CH faces challenges, and combining biochemical screening with genetic testing is crucial to improve screening sensitivity.

CH is mainly caused by the underdevelopment of the thyroid gland, dyshormonogenesis, and central congenital hypothyroidism. Therefore, we selected the hotspot mutant genes *DUOX2* and *DUOXA2* in CH in the Chinese population, which are associated with disorders of thyroid hormone synthesis and secretion. The TSHR gene, which is associated with thyroid dysplasia, was also selected; TSH resistance caused by loss-of-function mutations in *TSHR* is the most common genetic factor leading to congenital hypothyroidism. In addition, central congenital hypothyroidism is strongly associated with variants in the *PROP1* gene, which is usually associated with pituitary hormone underproduction. In particular, combined pituitary hormone deficiency caused by mutations in the *PROP1* gene is associated with decreased TSH secretion [28]. Although CH screening is primarily aimed at primary hypothyroidism, testing for *PROP1* gene mutations may reveal some undetected cases of secondary hypothyroidism [29]. The inclusion of the *PROP1* gene in the range of tests is aimed at further research into potential genetic factors. Although this is not the main purpose of the screening, it undoubtedly provides extremely valuable genetic information. With respect to the relationship between genotype and biochemical phenotype, significant differences were observed in sFT4 levels between patients with and without gene variants and, compared with the patients in the no mutation group, most patients with gene variants had lower FT4 levels. *DUOX2* is involved mainly in the production of peroxide protein complexes and catalyzes the synthesis of thyroid hormones in thyroid follicular cells [30]. The protein encoded by *DUOX2* is involved in the iodization process of thyroxine synthesis, which is closely related to the generation of FT4 [31]. In this study, all genes were merged with one or two *DUOX2* mutations. These children exhibit various clinical manifestations, including CH and HTT, indicating that mutations in *DUOX2* are not necessarily related to clinical classification [31]. Our research revealed that homozygous mutations or compound heterozygous mutations of c.1588A>T and c.3329G>A lead to abnormal thyroid function, but the c.1588A>T and c.3329G>A variants had no correlation with clinical classification (CH or HTT). In addition, the c.1588A>T variant was related to sFT4 levels, indicating that patients with the c.1588A>T variant have more severe hypothyroidism. The damage to the functional domain caused by the c.1588A>T variant is related to peroxidase-like activity [32,33]. The region where c. 1588A>T is located in the main functional domain for *DUOX2* to exert peroxidase activity. The above results indicate that *DUOX2* mutations in major structural domains, such as the peroxidase-like region, have a significant effect on thyroid function. Studies have shown that mutations in *DUOX2* can initiate CH, but the clinical phenotype, primarily the manifestation of transient congenital hypothyroidism, is variable [34,35]. Our study had a short follow-up period, and the clinical phenotypic data were not comprehensive. Variable phenotypes are presumed to be caused by other undetected genetic mutations, individual differences, stochastic phenomena, or environmental factors [32,36]. With regards to *DUOX2* over 100 different mutations have been reported but there is ambiguity about the number of these which are truly pathogenic. Therefore, pathogenic gene screening for patients with CH, enrichment of the mutation spectrum, and identification of the relationships between gene mutations and phenotypes are the recommended steps to achieve accurate clinical diagnosis and treatment of CH.

Although we performed relatively systematic and comprehensive screening for candidate genes associated with CH, some limitations should be considered when reviewing our findings. First, this is a highly selected population, and a larger sample size study is needed to further confirm the correlation between gene mutations and clinical phenotypes; future investigations should expand the scope of genetic testing to include a larger number of genes associated with hypothyroidism. Second, most of the patients had a short follow-up period, and the clinical phenotypic data were not comprehensive. Therefore, it is not analytically conducive to determine the correlation between the detected mutation and the clinical phenotype. Finally, it is recommended that the variants identified in this study be

subjected to in vitro functional studies. The subsequent studies will perform and analyze three-dimensional models of the missense variants identified in the *DUOX2* gene, with a particular focus on one of the most prevalent variants, p.Arg1110Gln, among others. Therefore, further studies are needed to expand the mutation spectrum of CH and to verify the functions of the associated mutations, which may provide more profound insight into the etiology of CH.

This study reveals that the latest incidence rate of hypothyroidism in China is 1/315 (including CH and HTT) by using routine screening and gene screening methods and provides a basis for research on neonatal CH genetic screening in China. Genetic screening is a supplement to biochemical screening. The incorporation of gene screening and traditional biochemical screening into neonatal CH screening is conducive to avoiding missed diagnoses. The advantages of this study include that it is the first relatively large multicenter study on the genetic diagnosis of children with CH in China, with a comprehensive clinical process for analysis through genetic diagnosis. However, the strategy of CH gene screening is still in the exploration stage, and the genes and loci covered by CH gene screening in Chinese newborns need to be further evaluated. Moreover, our first systematic and comprehensive screening of four CH-candidate genes in Chinese newborns preliminarily proved the necessity, feasibility, and importance of clinical gene screening for neonates and revealed that the main mutated gene of the four genes tested in this study was *DUOX2*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijns10040078/s1, Table S1: Normal cut-off value range of thyroid function index; Table S2: Clinical Information, and detected variants of studied patients with CH. Table S3: Clinical Information, and detected variants of studied patients with HTT.

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Institutional Review Board Statement: The study was conducted according to the principles expressed in the Declaration of Helsinki. The study design and protocol were reviewed and approved by the Ethics Committee of Xinhua Hospital Affiliated with Shanghai Jiaotong University School of Medicine (approval number: (2020)CJ0993, approval date: 22 December 2020).

Informed Consent Statement: Informed consent was obtained from parents or any legal representatives after a full explanation of the purpose and nature of all the procedures used.

Data Availability Statement: Access to the data included in the analyses can be provided upon request to the corresponding author.

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References

1. Rastogi, M.V.; LaFranchi, S.H. Congenital hypothyroidism. *Orphanet J. Rare Dis.* **2010**, *5*, 17. [CrossRef] [PubMed]
2. Deng, K.; He, C.; Zhu, J.; Liang, J.; Li, X.; Xie, X.; Yu, P.; Li, N.; Li, Q.; Wang, Y. Incidence of congenital hypothyroidism in China: Data from the national newborn screening program, 2013–2015. *J. Pediatr. Endocrinol. Metab.* **2018**, *31*, 601–608. [CrossRef] [PubMed]
3. Yu, B.; Long, W.; Yang, Y.; Wang, Y.; Jiang, L.; Cai, Z.; Wang, H. Newborn Screening and Molecular Profile of Congenital Hypothyroidism in a Chinese Population. *Front. Genet.* **2018**, *9*, 509. [CrossRef] [PubMed]
4. Cherella, C.E.; Wassner, A.J. Congenital hypothyroidism: Insights into pathogenesis and treatment. *Int. J. Pediatr. Endocrinol.* **2017**, *2017*, 11. [CrossRef] [PubMed]
5. Berry, S.A. Newborn screening. *Clin. Perinatol.* **2015**, *42*, 441–453. [CrossRef]
6. Keskinkilic, B. Neonatal screening programs. *Clin. Biochem.* **2014**, *47*, 692. [CrossRef]
7. Peters, C.; van Trotsenburg, A.S.P.; Schoenmakers, N. Diagnosis of endocrine disease: Congenital hypothyroidism: Update and perspectives. *Eur. J. Endocrinol.* **2018**, *179*, R297–R317. [CrossRef] [PubMed]
8. McMahon, R.; DeMartino, L.; Sowizral, M.; Powers, D.; Tracy, M.; Caggana, M.; Tavakoli, N.P. The Impact of Seasonal Changes on Thyroxine and Thyroid-Stimulating Hormone in Newborns. *Int. J. Neonatal Screen.* **2021**, *7*, 8. [CrossRef]
9. Cavarzere, P.; Camilot, M.; Popa, F.I.; Lauriola, S.; Teofoli, F.; Gaudino, R.; Vincenzi, M.; Antoniazzi, F. Congenital hypothyroidism with delayed TSH elevation in low-birth-weight infants: Incidence, diagnosis and management. *Eur. J. Endocrinol.* **2016**, *175*, 395–402. [CrossRef]
10. McGrath, N.; Hawkes, C.P.; Mayne, P.; Murphy, N.P. Optimal Timing of Repeat Newborn Screening for Congenital Hypothyroidism in Preterm Infants to Detect Delayed Thyroid-Stimulating Hormone Elevation. *J. Pediatr.* **2019**, *205*, 77–82. [CrossRef]
11. Stoupa, A.; Kariyawasam, D.; Polak, M.; Carre, A. Genetics of congenital hypothyroidism: Modern concepts. *Pediatr. Investig.* **2022**, *6*, 123–134. [CrossRef] [PubMed]
12. Nagasaki, K.; Minamitani, K.; Nakamura, A.; Kobayashi, H.; Numakura, C.; Itoh, M.; Mushimoto, Y.; Fujikura, K.; Fukushi, M.; Tajima, T. Guidelines for Newborn Screening of Congenital Hypothyroidism (2021 Revision). *Clin. Pediatr. Endocrinol.* **2023**, *32*, 26–51. [CrossRef] [PubMed]
13. Tong, F.; Wang, J.; Xiao, R.; Wu, B.B.; Zou, C.C.; Wu, D.W.; Wang, H.; Zou, H.; Han, L.S.; Yang, L.; et al. Application of next generation sequencing in the screening of monogenic diseases in China, 2021: A consensus among Chinese newborn screening experts. *World J. Pediatr.* **2022**, *18*, 235–242. [CrossRef]
14. Guo, Y.; Jiang, J.; Xu, Z. Chinese genetic variation database of inborn errors of metabolism: A systematic review of published variants in 13 genes. *Orphanet J. Rare Dis.* **2023**, *18*, 148. [CrossRef] [PubMed]
15. Stephenson, A.; Lau, L.; Eszlinger, M.; Paschke, R. The Thyrotropin Receptor Mutation Database Update. *Thyroid* **2020**, *30*, 931–935. [CrossRef]
16. Subspecialty Group of Endocrinologic H; Metabolic Diseases TSoPCMA; Group for Newborn Screening SoCHCPMA. Consensus statement on the diagnosis and management of congenital hypothyroidism. *Zhonghua Er Ke Za Zhi* **2011**, *49*, 421–424.
17. Richards, S.; Aziz, N.; Bale, S.; Bick, D.; Das, S.; Gastier-Foster, J.; Grody, W.W.; Hegde, M.; Lyon, E.; Spector, E.; et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **2015**, *17*, 405–424. [CrossRef]
18. Riggs, E.R.; Andersen, E.F.; Cherry, A.M.; Kantarci, S.; Kearney, H.; Patel, A.; Raca, G.; Ritter, D.I.; South, S.T.; Thorland, E.C.; et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet. Med.* **2020**, *22*, 245–257. [CrossRef]
19. Yao, Y.; Deng, K.; Zhu, J.; Xiang, L.; Yuan, X.; Li, Q.; Liu, L.; Xu, W. Increased incidence of congenital hypothyroidism in China: An analysis of 119 million screened newborns. *Eur. J. Pediatr.* **2023**. *ahead of print*. [CrossRef]
20. Tan, M.; Jiang, X.; Mei, H.; Feng, Y.; Xie, T.; Tang, C.; Chen, Q.; Zeng, C.; Huang, Y. Incidence tendency, etiological classification and outcome of congenital hypothyroidism in Guangzhou, China: An 11-year retrospective population-based study. *J. Pediatr. Endocrinol. Metab.* **2022**, *35*, 1141–1146. [CrossRef]
21. Yao, Y.; Yuan, X.; Zhu, J.; Xiang, L.; Li, Q.; Deng, K.; Li, X.; Liu, H. Geographic variations in the incidence of congenital hypothyroidism in China: A retrospective study based on 92 million newborns screened in 2013–2018. *Chin. Med. J.* **2021**, *134*, 2223–2230. [CrossRef] [PubMed]
22. Chiesa, A.E.; Tellechea, M.L. Update on Neonatal Isolated Hyperthyrotropinemia: A Systematic Review. *Front. Endocrinol.* **2021**, *12*, 643307. [CrossRef]
23. Cuestas, E.; Gaido, M.I.; Capra, R.H. Transient neonatal hyperthyrotropinemia is a risk factor for developing persistent hyperthyrotropinemia in childhood with repercussion on developmental status. *Eur. J. Endocrinol.* **2015**, *172*, 483–490. [CrossRef] [PubMed]
24. Chen, J.; Lin, S.; Zeng, G.; Wang, W.; Lin, Z.; Xu, C.; He, Y.; Shi, J.; Zhou, X.; Niu, C.; et al. Epidemiologic Characteristics and Risk Factors for Congenital Hypothyroidism from 2009 To 2018 in Xiamen, China. *Endocr. Pract.* **2020**, *26*, 585–594. [CrossRef]
25. Zheng, X.; Ma, S.; Guo, M.; Qiu, Y.; Yang, L. Compound Heterozygous Mutations in the DUOX2/DUOXA2 Genes Cause Congenital Hypothyroidism. *Yonsei Med. J.* **2017**, *58*, 888–890. [CrossRef] [PubMed]

26. Ye, L.; Yin, Y.; Chen, M.; Gong, N.; Peng, Y.; Liu, H.; Miao, J. Combined genetic screening and traditional newborn screening to improve the screening efficiency of congenital hypothyroidism. *Front. Pediatr.* **2023**, *11*, 1185802. [CrossRef] [PubMed]
27. Chen, T.; Fan, C.; Huang, Y.; Feng, J.; Zhang, Y.; Miao, J.; Wang, X.; Li, Y.; Huang, C.; Jin, W.; et al. Genomic Sequencing as a First-Tier Screening Test and Outcomes of Newborn Screening. *JAMA Netw. Open.* **2023**, *6*, e2331162. [CrossRef]
28. Schoenmakers, N.; Alatzoglou, K.S.; Chatterjee, V.K.; Dattani, M.T. Recent advances in central congenital hypothyroidism. *J. Endocrinol.* **2015**, *227*, R51–71. [CrossRef]
29. van Trotsenburg, P.; Stoupa, A.; Léger, J.; Rohrer, T.; Peters, C.; Fugazzola, L.; Cassio, A.; Heinrichs, C.; Beauloye, V.; Pohlenz, J.; et al. Congenital Hypothyroidism: A 2020–2021 Consensus Guidelines Update-An ENDO-European Reference Network Initiative Endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. *Thyroid* **2021**, *31*, 387–419. [CrossRef]
30. De Deken, X.; Wang, D.; Many, M.C.; Costagliola, S.; Libert, F.; Vassart, G.; Dumont, J.E.; Miot, F. Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. *J. Biol. Chem.* **2000**, *275*, 23227–23233. [CrossRef]
31. Maruo, Y.; Nagasaki, K.; Matsui, K.; Mimura, Y.; Mori, A.; Fukami, M.; Takeuchi, Y. Natural course of congenital hypothyroidism by dual oxidase 2 mutations from the neonatal period through puberty. *Eur. J. Endocrinol.* **2016**, *174*, 453–463. [CrossRef] [PubMed]
32. Fu, C.; Zhang, S.; Su, J.; Luo, S.; Zheng, H.; Wang, J.; Qin, H.; Chen, Y.; Shen, Y.; Hu, X.; et al. Mutation screening of DUOX2 in Chinese patients with congenital hypothyroidism. *J. Endocrinol. Investig.* **2015**, *38*, 1219–1224. [CrossRef] [PubMed]
33. Yoshizawa-Ogasawara, A.; Ogikubo, S.; Satoh, M.; Narumi, S.; Hasegawa, T. Congenital hypothyroidism caused by a novel mutation of the dual oxidase 2 (DUOX2) gene. *J. Pediatr. Endocrinol. Metab.* **2013**, *26*, 45–52. [CrossRef] [PubMed]
34. Wang, H.; Kong, X.; Pei, Y.; Cui, X.; Zhu, Y.; He, Z.; Wang, Y.; Zhang, L.; Zhuo, L.; Chen, C.; et al. Mutation spectrum analysis of 29 causative genes in 43 Chinese patients with congenital hypothyroidism. *Mol. Med. Rep.* **2020**, *22*, 297–309. [CrossRef] [PubMed]
35. Long, W.; Zhou, L.; Wang, Y.; Liu, J.; Wang, H.; Yu, B. Complicated Relationship between Genetic Mutations and Phenotypic Characteristics in Transient and Permanent Congenital Hypothyroidism: Analysis of Pooled Literature Data. *Int. J. Endocrinol.* **2020**, *2020*, 6808517. [CrossRef] [PubMed]
36. Zheng, Z.; Yang, L.; Sun, C.; Wu, J.; Luo, F.; Zhou, W.; Lu, W. Genotype and phenotype correlation in a cohort of Chinese congenital hypothyroidism patients with DUOX2 mutations. *Ann. Transl. Med.* **2020**, *8*, 1649. [CrossRef] [PubMed]

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Review

Is It Time to Expand Newborn Screening for Congenital Hypothyroidism to Other Rare Thyroid Diseases?

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Abstract

Congenital hypothyroidism (CH) is a heterogeneous condition present at birth, resulting in severe-to-mild thyroid hormone deficiency. This condition is difficult to recognize shortly after birth. Therefore, many countries worldwide have implemented newborn screening (NBS) programs for CH since the 1970s. The most recent European guidelines strongly recommend screening for primary CH, as well as for central CH when financial resources are available. However, no consensus has been reached yet to screen more rare forms of CH, such as Allan–Herndon–Dudley syndrome (AHDS), an X-linked condition linked to mutations in the gene encoding a transmembrane monocarboxylate transporter (MCT8), resistance to thyroid hormone beta (RTHβ), and resistance to thyroid hormone alfa (RTHα). The combined measurement of thyroid-stimulating hormone (TSH) and total thyroxine (TT4) on DBS currently allows the recognition of central CH (TSH low/normal and low TT4 without defects in transport proteins). With the introduction of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for measurement of free triiodothyronine (FT3) and free thyroxine (FT4), it would be possible to screen for RTHβ (TSH normal/high and high FT4). More complicated would be the method to screen RTHα. It would require the combined measurement of FT4 and FT3 and the determination of FT3/FT4 ratio, while the combined measurement of FT3 and reverse T3 (rT3) to calculate FT3/rT3 ratio would be useful to screen AHDS. In this article, we provide some reflections on expanding NBS for primary CH also to other rare forms of CH.

Keywords: congenital hypothyroidism; newborn screening; rare diseases

1. Introduction

Congenital hypothyroidism (CH) is a heterogeneous condition present at birth, resulting in severe-to-mild thyroid hormone deficiency. CH may be caused by abnormal development or function of thyroid gland (primary CH), or by dysfunction of hypothalamus or pituitary gland (central CH), or more rarely by impaired transport or action of thyroid hormone at the tissue level, such as monocarboxylate transporter 8 defects or Alan–Herndon–Dudley syndrome, resistance to thyroid hormone alfa (RTHα) or resistance

to thyroid hormone beta (RTHβ) [1–3]. Thyroid hormone deficiency early in life is harmful for growth and neurocognitive development. The critical period for brain development occurs in the first three years of life, during which thyroid hormones play a key role.

Thyroid hormone deficiency is difficult to recognize shortly after birth. Therefore, many countries worldwide have implemented newborn screening (NBS) programs for CH since the 1970s [4–6]. The most recent European guidelines strongly recommend screening for primary CH. These guidelines also recommend screening for central CH when financial resources are available. However, no consensus has been reached yet to screen more rare forms of CH [1].

Here we present the Italian NBS program for CH as an example of longstanding, nationwide screening program for the disease and provide some considerations on the possibility of expanding this program to more rare forms of congenital thyroid diseases.

2. Italian NBS Program for CH

In Italy, a nationwide mandatory program of NBS for the identification and early treatment of CH, phenylketonuria, and cystic fibrosis was established in 1992 [7]. Since then, around 16 million infants have been screened in our country. More recently, an additional nationwide NBS program aimed at identifying more than 50 rare inherited metabolic diseases has been introduced [8]. This new program has reduced the window of age at screening to 48–72 h of life. The Italian NBS program for CH is supported by a nationwide surveillance system carried out by the Italian National Registry of Infants with CH (INRICH) at the Italian National Institute of Health [9].

Currently, 16 regional or inter-regional screening laboratories are active in our country to screen primary CH. Since measurement of TSH is the most sensitive test for detecting primary CH and according to the European guidelines [1], all the Italian laboratories use the measurement of TSH on dried blood spot (DBS) as the primary screening test to identify all forms of primary CH (mild, moderate, and severe). In all the laboratories, the measurement of TSH is performed by means of GSP Neonatal hTSH Time-Resolved Fluorescence assay (Revvity, Turku, Finland). A re-screening on DBS at 2 weeks of life is also performed in infants at risk of delayed TSH rise, i.e., infants with gestational age <37 weeks' gestation and/or low birth weight, with extra-thyroidal malformations, admitted to NICU, twins.

On first screening, the TSH threshold values used for referral (after confirmation of the screening test results) are variable, ranging 6–10 mIU/L blood according to the percentile of the TSH distribution chosen in each region (INRICH data). However, if the screening test is $\geq$40 mIU/L blood, the infant is immediately referred to the pediatric clinical center without waiting for confirmatory laboratory test, as this value is highly suggestive of moderate-to-severe primary CH [1]. Only in the Campania region is the TSH threshold for an immediate referral to the pediatric clinical center $\geq$20 mIU/L blood. On re-screening (2 weeks of life), the TSH threshold levels are also variable, ranging 3–9 mIU/L blood (INRICH data).

Over the years, the increased assay sensitivity and a more effective analysis of the distribution of TSH in the screened neonatal population have led to lower TSH cutoffs in some screening programs, including the Italian NBS program for CH [10].

Lowering of TSH screening cutoffs along with other factors, such as increased survival rate of preterm babies and twin births, changes in screening population demographics, and a progressive adoption of re-screening strategy in infants at risk of delayed TSH rise [11–17], have led to an increased incidence of primary CH. Currently in Italy, the estimated incidence of primary CH is 1:1100 live births (www.iss.it/rnic accessed on 18 August 2025). This incidence is similar to that recently reported in other countries using similar NBS strategies for CH [18–21].

The most recent European guidelines recommend adding measurement of total (TT4) or free T4 (FT4) to TSH to screen for central CH [1]. At present in Italy, only 3 regions

(Campania, Calabria, Sardinia) have adopted a screening strategy employing TSH + TT4 testing to detect both primary and central CH. TT4 is measured by means of GSP Neonatal hT4 Time-Resolved Fluorescence assay. In these regions, the blood TSH threshold values used on the first screening are set at the 97.5th percentile of the distribution, corresponding to 6.0 mIU/L in Campania, 7.0 mIU/L in Calabria, and 5.5 mIU/L in the Sardinia region; whereas the blood TT4 threshold value is set at the 0.15th percentile in Campania (4.2 μg/dL), the 2.5th percentile in Calabria (7.0 μg/dL), and the 2.2nd percentile in the Sardinia region (6.0 μg/dL). On re-screening (2 weeks of life), the blood TSH threshold values for referral are 5.0 mIU/L in Campania, 3.0 mIU/L in Calabria, and 5.5 mIU/L in the Sardinia region, while the blood TT4 threshold is ≤4.0 μg/dL in all the three regions. The risk of false-positive results, deriving from preterm and sick neonates often showing low TT4 with non-elevated TSH levels [22], is overcome by the re-screening procedure at 2 weeks of age.

It is evident that these regional screening programs testing for both primary and central CH are expensive, and an optimization process is necessary to make these programs cost-effective in terms of costs and workload. This process could include the introduction of the routinary measurement of thyroxine-binding globulin (TBG) with the use of specific reference intervals to rule out cases of complete and partial TBG deficit [6].

Although epidemiological data on the incidence of central CH in these regions are not yet available, it can be assumed that the diagnosis of central CH may be delayed in the other Italian regions.

3. Central CH

Central CH results from a dysfunction of the hypothalamus or pituitary gland, leading to inadequate stimulation of a normal thyroid. In comparison with primary CH, central CH can be more difficult to diagnose because it does not present with elevated TSH levels, resulting in the main "false-negative" condition by the TSH screening test. The diagnosis of central CH is most often based on finding low or normal serum FT4 associated with an inappropriately low or normal serum TSH concentration. It is important to be aware of confounding factors and other conditions that may present with a similar biochemical picture (e.g., TBG defects or Non-Thyroidal Illness Syndrome, etc.) [23].

Central CH is mainly diagnosed in patients with hypothalamic–pituitary disorders, usually in combination with other pituitary hormone defects. In these cases, the diagnosis of central hypothyroidism is facilitated by the presence of symptoms and signs that are typical of other hormone deficiencies (severe hypoglycemia, presence of micropenis, etc.), whereas the isolated presentation of central CH is difficult to diagnose, because of frequently mild manifestations. Approximately 90 percent of cases of isolated central congenital CH are due to genetic defects. Depending on the cause of the disease, the TSH secretion deficit may be quantitative (reduced number of TSH-secreting cells) or qualitative (altered bioactivity of secreted TSH) [23–25].

Congenital forms of central hypothyroidism mostly result from structural lesions such as pituitary hypoplasia, Rathke's pouch cysts, or midline defects, which mainly cause quantitative defects in TSH secretion. However, functional defects in TSH biosynthesis and/or release are caused by loss-of-function mutations in genes encoding various types of genes, such as *TRHR, TSHβ*, *IGSF1, TBL1X,* or *IRS4* as candidates for isolated central CH or *POU1F1, PROP1, HESX1, LHX3, LHX4, SOX2, SOX3, OTX2*, and *LEPR* as candidates for combined pituitary hormone defects (CPHDs) [1,2,23,26,27]. The mode of inheritance of these defects is variable (autosomal recessive or X-linked inheritance in most cases, but dominant inheritance was described in rare cases of combined pituitary hormone defects).

The reported incidence of central CH detected by neonatal screening varies between 1:30,000 and 1:16,000, depending on the adopted screening strategy [28–30]. Early diagnosis of

central CH is particularly crucial for growth and neurocognitive development because of the significant impact of thyroid hormone deficiency. Infants with undiagnosed and untreated central CH are at high risk of severe neurodevelopmental delays, including cognitive impairment, motor disabilities, and an overall reduced quality of life. Therefore, as with primary CH, neonatal screening for central CH can also allow an early diagnosis and hence the early initiation of treatment with levothyroxine, which may improve the neurological outcome [2,31].

Combined measurement of TSH and T4 levels in newborn screening can help effectively identify central CH. Early diagnosis not only improves individual health outcomes but also has wider socio-economic benefits. By preventing severe intellectual disability and other health complications, early treatment reduces the long-term healthcare costs associated with managing untreated central CH. Families benefit from reduced emotional and financial burden, and society benefits from the improved productivity and quality of life of individuals who receive timely and appropriate treatment. In addition, the neonatal diagnosis of central hypothyroidism is also effective in making an earlier diagnosis and treatment of multiple pituitary hormone deficiencies, commonly associated with central CH, thus preventing life-threatening conditions [32,33].

Screening for central CH is complicated because the measurement of T4 is influenced by several factors including binding proteins, the clinical condition of the patient, gestational age, and the individual setpoint of the hypothalamic–pituitary–thyroid (HPT) axis [6]. The Netherlands provides an effective newborn screening strategy for central CH. Since 1995, this program has consisted of a three-step approach, with T4 measurement in all newborns as the first step, TSH measurement in the lowest 20% of T4 concentrations, and thyroxine-binding globulin (TBG) measurement in the lowest 5% to prevent false-positive results due to TBG deficiency [6,31]. In this screening program, the calculation of T4/TBG ratio, as an indirect measure for FT4, has effectively lowered the number of false-positive referrals essentially due to infants with complete TBG deficiency. In addition, reference intervals (RIs) for the NBS parameters—total T4, TSH, TBG, and total T4/TBG ratio—have been recently established in the Dutch NBS, so that the use of the TBG reference intervals to identify partial TBG deficiency has further reduced false-positive referrals by approximately 50%. As a result, the Dutch screening algorithm has been adapted to exclude total and partial TBG deficiency (TBG < 105 nmol/L blood) and this led to an improvement in the positive predicted value (currently 21%) while maintaining the current sensitivity of central CH detection [34,35]. It is worth noting that the current three-step T4-reflex TSH-reflex TBG NBS program also led to an improved detection of central CH with an incidence of 1:16,404, which appears to be much higher than that reported in countries using T4-reflex TSH or TSH-based strategies [32]. Regarding primary CH, it is important to underline that this three-step Dutch NBS program effectively detects severe forms of primary CH, with a positive predictive value (PPV) of 55% [34]. Nevertheless, as neonates at risk of primary CH are preselected based on their T4 concentrations (80% of all neonates do not receive a TSH measurement), mild primary CH cases with normal T4 concentrations might be missed. This could contribute to the lower incidence of primary CH in the Netherlands compared to other countries [5,34–36].

In Italy, the lack of a uniform NBS program for central CH may lead to delayed diagnosis in different regions, highlighting the need for a nationwide implementation of comprehensive screening protocols. The strategy of using TSH + T4 as primary screening tests, along with TBG measurement and adoption of specific reference intervals, appears to be the most effective strategy to adopt in our country for an effective detection of both primary and central CH on a large scale and at an acceptable cost for our health system. It would eliminate regional disparities and ensure that all newborns with central CH have equal access to early diagnosis and treatment.

4. Other Rare Congenital Thyroid Diseases

4.1. MCT8 Defects

Allan–Herndon–Dudley syndrome (AHDS) is an X-linked condition prevalently affecting boys, which is associated with severe intellectual and motor disabilities [37]. AHDS was described more than 60 years ago, but only in 2004 was this disease linked to mutations in *SLC16A2*, the gene encoding the transmembrane monocarboxylate transporter 8 (MCT8). This gene is crucial in the transport of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) into several tissues, including the brain [38–40]. The estimated incidence of MCT8 defects is around 1:70,000 newborns [38,39]. However, this incidence may be underestimated, and many cases are left underdiagnosed due to the current absence of a recognizable biochemical signature.

As shown in Table 1, the disease is biochemically characterized by high levels of serum free T3 (FT3), low or borderline low levels of serum FT4, low serum reverse T3 (rT3), and a TSH concentration in the normal range [41]. Although these biochemical findings are suggestive of a thyroid hormone disturbance, the clinical presentation observed in AHDS includes both "thyrotoxic" peripheral tissues (bone, heart, skeletal muscles, liver) and the "hypothyroid" brain [37]. Interestingly, the degree of psychomotor impairment is similar or even worse compared to that seen in untreated primary CH. Clinical trials have been recently conducted to understand the efficacy and safety of treatment with a triiodothyronine analog named TRIAC (triiodothyroacetic acid) [42]. This treatment is revealed to be effective in improving peripheral metabolic conditions. Nevertheless, it has almost no effect on psychoneuromotor deficits because of the delayed start of treatment. In this regard, a recent study has shown that prenatal intraamniotic thyroxine treatment improved the neuromotor and neurocognitive function in a boy with MCT8 deficiency born to a carrier mother, thus showing that prenatal start of treatment with thyroid hormone analogs can rescue at least part of the phenotype [43].

Table 1. Molecular and biochemical signature, treatment, and screening tests in rare congenital thyroid disorders.

Molecular and Biochemical Signature and Treatment	**Central Hypothyroidism**	**Resistance to Thyroid Hormone Beta (RTHβ)**	**Resistance to Thyroid Hormone Alpha (RTHα)**	**Monocarboxylate Transporter 8 (MCT8) Deficiency**
Gene	*several*	*THRB*	*THRA*	*SLC16A2*
Inheritance pattern	Variable *	Dominant	Dominant	X-linked
Serum Free T4	low	high	low-normal or low	low-normal or low
Serum Free T3	low or normal	high	high-normal or high	high or high-normal
Serum Reverse T3	--	--	--	low
Serum TSH	low or normal (rarely, mildly raised)	normal or high	normal (rarely, mildly raised)	normal (rarely, mildly raised)
Treatment	LT4	TRIAC	LT4	TRIAC
Screening tests on DBS and expected results				
Current Screening tests	TSH + TT4	--	--	--
Possible future screening tests **		TSH + FT4	TSH, FT4 + FT3, and calculation of FT3/FT4 ratio	TSH, FT3, rT3, and calculation of FT3/rT3 ratio
Expected results of screening tests	low/normal TSH and low TT4	normal/high TSH and high FT4	high FT3/FT4 ratio	high FT3/rT3 ratio

* List of candidate genes for central CH: TRHR (recessive), TSHβ (recessive), IGSF1 (X-linked), TBL1X (X-linked), IRS4 (X-linked), LEPR (recessive), POU1F1 (recessive or dominant), PROP1 (recessive), HESX1 (recessive or dominant), LHX3 (recessive), LHX4 (recessive or dominant), SOX2 (dominant), SOX3 (X-linked), OTX2 (dominant).
** Measurement of FT3, FT4, and rT3 performed by means of LC-MS/MS.

4.2. Resistance to Thyroid Hormone Beta

The key characteristic of RTHβ is a combination of raised thyroid hormones with non-suppressed TSH (Table 1) [44]. RTHβ patients can exhibit features of hyperthyroidism or hypothyroidism, reflecting either compensated hormone resistance in TRβ-expressing tissues (e.g., liver, pituitary) or approximately normal sensitivity to high circulating thyroid hormones in TRβ-expressing tissues (e.g., heart, brain). The condition is dominantly inherited and co-segregates with dominant negative heterozygous mutations in *THRB*, the gene encoding for thyroid receptor β (TRβ) [45]. Neurocognitive manifestations of RTHβ are anxiety and sleep disturbance, attention-deficit hyperactivity disorder [46,47], variable intellectual disability (lower nonverbal intelligence), language difficulties [48], and poor educational outcome [49]. Severe intellectual disability and cochlear dysfunction are characteristic of homozygous cases [50,51]. Recently reported data show that RTHβ patients are at significantly higher risk of major cardiovascular events (atrial fibrillation, myocardial infarction, heart failure) and of earlier mortality [52,53]. Inhibition of TSH secretion into lower circulating T4 and T3 obtained by treatment with TRIAC appears efficient in controlling thyrotoxic manifestations in adults and children with RTHβ [54,55]. An improvement in some neurological manifestations has also been observed, but clinical trials are required to understand if early (neonatal) start of TRIAC treatment can rescue the neurocognitive manifestations and/or reduce the adverse cardiovascular manifestations of the disease [54,56,57]. The incidence of RTHβ has been estimated to range between 1:40,000 and 1:19,000 neonates in the USA and Spain, respectively, by the finding from neonatal screening of raised total T4 associated with normal TSH levels [58,59].

4.3. Resistance to Thyroid Hormone Alfa

RTHα is a rare disorder, with less than 100 affected individuals reported to date. Although the phenotype is highly variable, many patients exhibit similar clinical features, including psychomotor delay (cognitive impairment, delayed growth milestones, dyspraxia) and peripheral manifestations. The RTHα phenotype recapitulates most of the features of untreated congenital hypothyroidism except the typical biochemical signature. The thyroid function tests are similar to those seen in AHDS or central hypothyroidism: TSH is generally within the reference range (rarely borderline elevated), FT4 is borderline low, and FT3 is borderline high, and this is hampering the diagnostic possibility of this condition (Table 1).

The disease is caused by dominant negative heterozygous mutations in *THRA*, the gene encoding for TRα [60,61]. The identified mutations lie in the same hot spots in the T3-binding domain of the receptor and the molecular mechanisms underlying this disease are similar to those previously described for RTHβ. To date, levothyroxine is the main treatment described for RTHα [62,63].

Although data are restricted to case reports or case series, thyroxine treatment in RTHα seems safe and well tolerated and provides beneficial effects for most patients [63–65]. The therapeutic responses are variable, depending upon the degree of resistance generated by the mutation and the age of treatment start. The neurocognitive outcome can be predicted to significantly improve or even normalize if the treatment could be started in the neonatal period (or even prenatally) for patients with mutant receptors that have a diminished, but not abolished, T3-binding affinity. The frequency of RTHα is unknown and underestimated at present because of the lack of a clear-cut biochemical signature, but its incidence can be expected to be similar to that of RTHβ, since both diseases are caused by dominant negative variants occurring in the same hot spots within the T3-binding domains, which are highly conserved between the two receptors.

5. NBS for Rare Congenital Thyroid Diseases

The early recognition of these rare congenital thyroid diseases would allow the neonatal start of thyroid hormone treatment aiming to rescue, at least partially, the neurocognitive and developmental phenotypes of these dramatic diseases. Particularly, the combined measurement of TSH and TT4 on DBS would easily allow the recognition of central CH (TSH low/normal and low TT4 without defects in transport proteins) and RTHβ (TSH normal/high and high FT4). More complicated would be the method to screen RTHα and AHDS. This would require the combined measurement of FT3 and FT4 in DBS samples with the calculation of FT3/FT4 ratio (high) to detect cases of RTHα, while the combined measurement of FT3 and rT3 with the calculation of FT3/rT3 ratio (high) could be used to detect infants with AHDS (MCT8 deficiency).

It is well known that the main drawback in the determination of circulating thyroid hormones is the interference of defects in transport proteins [66]. To avoid any interfering effect, the measurement of free thyroid hormones using equilibrium dialysis or ultrafiltration combined with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is considered the most suitable approach for measurement of concentration of free thyroid hormones T3, T4, and rT3 [41,67]. Although LC-MS/MS technology is generally employed for second-tier tests to reduce the false-positive rate, in many countries it is currently applied to primary screening for some inherited metabolic diseases for which other methods, including genomic screening, are not adequate [68]. The measurement of thyroid hormones by means of LC-MS/MS is therefore feasible. We are confident that with reduction in cost of LC-MS/MS measurements, the routinary application of this technology to screen for rare congenital thyroid diseases will be possible in a reasonable time.

In countries where a NBS program for primary CH is active, early diagnosis and treatment have dramatically changed the life of affected children, avoiding severe neurocognitive sequelae of a disease that is still the most frequent endocrine disease in infancy, with a case in every 1100–1500 liveborns [5]. Central CH and resistance to thyroid hormones RTHα and RTHβ are more rare forms of congenital hypothyroidism but similarly harmful without an early diagnosis and treatment.

In 1968, Wilson and Jungner criteria to screen for a disease included the high frequency in the population of the disease to screen, as well as the availability of treatment for patients with a disease recognized early by screening [69]. The introduction of the expanded NBS for several metabolic rare diseases in Italy, as in other countries, has led to reconsidering these criteria, introducing the new concept that screening should identify *actionable* diseases, including treatable diseases. Actionable diseases are conditions where early interventions lead to health gain for newborns and where early diagnosis avoids the lengthy diagnostic odyssey [70]. This therefore implies that, although the incidence of some of these disorders is very low (<1:100,000) and some of them are untreatable, their early detection and treatment is nevertheless beneficial because they can prevent the onset of disease symptoms or delay disease progression, improving the quality of life of the newborn, their families, and society [70]. These new concepts can be easily applied to central CH, MCT8 defects, RTHα, and RTHβ as well.

In addition, the development of new technologies such as tandem mass spectrometry, which offers the possibility of screening for many conditions using a single DBS [71–74], makes feasible the measurement of FT4, FT3, and rT3 on DBS with the possibility of providing early diagnosis and preventing or ameliorating the long-term consequences of the rare congenital forms of hypothyroidism.

Next-generation sequencing (NGS) may also be a powerful tool for diagnosing rare congenital thyroid diseases. Its ability to analyze multiple genes simultaneously and

identify novel mutations makes it a powerful approach for improving diagnosis, treatment, and research in the field of rare congenital thyroid diseases [75].

6. Conclusions

We believe that the current Italian NBS program, which is successful in screening for primary CH, could be expanded to address regional disparities and to detect the broad spectrum of other rare congenital thyroid disorders.

Although carrying out such a comprehensive screening program would generate additional costs in terms of laboratory and clinical activities, a uniform national protocol where the measurement of FT3, FT4, and rT3 is added to TSH by means of LC-MS/MS could eliminate regional disparities and ensure that all newborns, including those with rare congenital thyroid diseases, have equal access to early diagnosis and treatment, improving individual health outcomes, and reducing long-term healthcare costs as well as the burden on families.

Expanding NBS programs for CH to include other rare congenital thyroid disorders is therefore a crucial step towards giving all newborns the best start in life. Early detection and intervention are key to preventing serious health problems and improving the overall quality of life of affected children. For this reason, further studies are necessary to discover new and more specific biomarkers that, along with TSH FT3, FT4, rT3, as well as NGS (comprehensive targeted NGS panels up to whole-genome screening), can help to develop an effective expanded NBS program for rare congenital thyroid diseases.

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Abbreviations

The following abbreviations are used in this manuscript:

AHDS	Alan–Herndon–Dudley Syndrome
CH	Congenital Hypothyroidism
FT4	Free Thyroxine
FT3	Free Triiodothyronine
MCT8	Transmembrane monocarboxylate transporter 8
NBS	Newborn Screening
RTHα	Resistance to Thyroid Hormone alfa
RTHβ	Resistance to Thyroid Hormone beta
TRIAC	Triiodothyroacetic acid
TSH	Thyroid-Stimulating Hormone
TT4	Total Thyroxine
T3	Triiodothyronine

References

1. van Trotsenburg, P.; Stoupa, A.; Léger, J.; Rohrer, T.; Peters, C.; Fugazzola, L.; Cassio, A.; Heinrichs, C.; Beauloye, V.; Pohlenz, J.; et al. Congenital Hypothyroidism: A 2020–2021 Consensus Guidelines Update-An ENDO-European Reference Network Initiative Endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. *Thyroid* **2021**, *31*, 387–419. [CrossRef] [PubMed]
2. Persani, L.; Brabant, G.; Dattani, M.; Bonomi, M.; Feldt-Rasmussen, U.; Fliers, E.; Gruters, A.; Maiter, D.; Schoenmakers, N.; van Trotsenburg, A.S.P. 2018 European Thyroid Association (ETA) Guidelines on the Diagnosis and Management of Central Hypothyroidism. *Eur. Thyroid. J.* **2018**, *7*, 225–237. [CrossRef]
3. Persani, L.; Rodien, P.; Moran, C.; Visser, W.E.; Groeneweg, S.; Peeters, R.; Refetoff, S.; Gurnell, M.; Beck-Peccoz, P.; Chatterjee, K. 2024 European Thyroid Association Guidelines on diagnosis and management of genetic disorders of thyroid hormone transport, metabolism and action. *Eur. Thyroid. J.* **2024**, *13*, e240125. [CrossRef] [PubMed]
4. Cheetham, T.; Wood, C. Pediatric thyroid disease. *Clin. Endocrinol.* **2024**, *101*, 223–233. [CrossRef] [PubMed]
5. Grob, F.; Lain, S.; Olivieri, A. Newborn screening for primary congenital hypothyroidism: Past, present and future. *Eur. Thyroid. J.* **2025**, *14*, e240358. [CrossRef]
6. Garrelfs, M.R.; Mooij, C.F.; Boelen, A.; van Trotsenburg, A.S.P.; Zwaveling-Soonawala, N. Newborn screening for central congenital hypothyroidism: Past, present and future. *Eur. Thyroid. J.* **2025**, *14*, e240329. [CrossRef]
7. Italia. Legge 5 Febbraio 1992, n. 104. Legge-Quadro per L'assistenza, L'integrazione Sociale e i Diritti Delle Persone Handicappate. GU Serie Generale n. 39, 17 Febbraio 1992 (Suppl. Ordinario n. 30). Available online: www.gazzettaufficiale.it/eli/id/1992/02/17/092G0108/sg (accessed on 5 August 2025).
8. Italia. Legge 19 Agosto 2016, n. 167. Disposizioni in Materia di Accertamenti Diagnostici Neonatali Obbligatori per la Prevenzione e la cura Delle Malattie Metaboliche Ereditarie. (16G00180). GU Serie Generale n. 203, 31 Agosto 2016. Available online: www.gazzettaufficiale.it/eli/id/2016/08/31/16G00180/sg (accessed on 18 August 2025).
9. Olivieri, A.; The Study Group for Congenital Hypothyroidism. The Italian National Register of infants with congenital hypothyroidism: Twenty years of surveillance and study of congenital hypothyroidism. *Ital. J. Pediatr.* **2009**, *35*, 2. [CrossRef]
10. Olivieri, A.; Corbetta, C.; Weber, G.; Vigone, M.C.; Fazzini, C.; Medda, E. Congenital hypothyroidism due to defects of thyroid development and mild increase of TSH at screening: Data from the Italian National Registry of infants with congenital hypothyroidism. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 1403–1408. [CrossRef]
11. Olivieri, A.; Fazzini, C.; Medda, E.; Italian Study Group for Congenital Hypothyroidism. Multiple factors influencing the incidence of congenital hypothyroidism detected by neonatal screening. *Horm. Res. Paediatr.* **2015**, *83*, 86–93. [CrossRef]
12. Medda, E.; Vigone, M.C.; Cassio, A.; Calaciura, F.; Costa, P.; Weber, G.; de Filippis, T.; Gelmini, G.; Di Frenna, M.; Caiulo, S.; et al. Neonatal Screening for Congenital Hypothyroidism: What Can We Learn From Discordant Twins? *J. Clin. Endocrinol. Metab.* **2019**, *104*, 5765–5779. [CrossRef]
13. Caiulo, S.; Corbetta, C.; Di Frenna Met, a.l. Newborn screening for congenital hypothyroidism: The benefit of using differential cutoffs in a 2-screen program. *J. Clin. Endocrinol. Metab.* **2021**, *106*, e338–e349. [CrossRef]
14. Hinton, C.F.; Harris, K.B.; Borgfeld, L.; Drummond-Borg, M.; Eaton, R.; Lorey, F.; Therrell, B.L.; Wallace, J.; Pass, K.A. Trends in incidence rates of congenital hypothyroidism related to select demographic factors: Data from the United States, California, Massachusetts, New York, and Texas. *Pediatrics* **2010**, *125* (Suppl. S2), S37–S47. [CrossRef] [PubMed]
15. Fisher, D.A. Thyroid system immaturities in very low birth weight premature infants. *Semin. Perinatol.* **2008**, *32*, 387–397. [CrossRef] [PubMed]
16. Larson, C.; Hermos, R.; Delaney, A.; Daley, D.; Mitchell, M. Risk factors associated with delayed thyrotropin elevations in congenital hypothyroidism. *J. Pediatr.* **2003**, *143*, 587–591. [CrossRef]
17. Hyman, S.J.; Greig, F.; Holzman, I.; Patel, A.; Wallach, E.; Rapaport, R. Late rise of thyroid stimulating hormone in ill newborns. *J. Pediatr. Endocrinol. Metab.* **2007**, *20*, 501–510. [CrossRef] [PubMed]
18. Nagasaki, K.; Sato, H.; Sasaki, S.; Nyuzuki, H.; Shibata, N.; Sawano, K.; Hiroshima, S.; Asami, T. Re-evaluation of the prevalence of permanent congenital hypothyroidism in Niigata, Japan: A retrospective study. *Int. J. Neon Screeen* **2021**, *7*, 27. [CrossRef]
19. McGrath, N.; Hawkes, C.P.; McDonnell, C.M.; Cody, D.; O'Connell, S.M.; Mayne, P.D.; Murphy, N.P. Incidence of congenital hypothyroidism over 37 years in Ireland. *Pediatrics* **2018**, *142*, e20181199. [CrossRef]
20. Chen, J.; Lin, S.; Zeng, G.; Wang, W.; Lin, Z.; Xu, C.; He, Y.; Shi, J.; Zhou, X.; Niu, C.; et al. Epidemiologic characteristics and risk factors for congenital hypothyroidism from 2009 to 2018 in Xiamen, China. *Endocr. Pract.* **2020**, *26*, 585–594. [CrossRef]
21. Gunnerbeck, A.; Lundholm, C.; von Döbeln, U.; Zetterström, R.H.; Almqvist, C.; Nordenström, A. Neonatal screening for congenital hypothyroidism in Sweden 1980–2013: Effects of lowering the thyroid-stimulating hormone threshold. *Eur. J. Endocrinol.* **2023**, *188*, 536–546. [CrossRef]

22. Rose, S.R.; Blunden, C.E.; Jarrett, O.O.; Kaplan, K.; Caravantes, R.; Akinbi, H.T. Utility of repeat testing for congenital hypothyroidism in infants with very low birth weight. *J. Pediatr.* **2022**, *242*, 152–158.e1. [CrossRef]
23. Feldt-Rasmussen, U.; Effraimidis, G.; Klose, M. The hypothalamus-pituitary-thyroid (HPT)-axis and its role in physiology and pathophysiology of other hypothalamus-pituitary functions. *Mol. Cell Endocrinol.* **2021**, *525*, 111173. [CrossRef] [PubMed]
24. Beck-Peccoz, P.; Persani, L. Variable biological activity of thyroid-stimulating hormone. *Eur. J. Endocrinol.* **1994**, *131*, 331–340. [CrossRef] [PubMed]
25. Persani, L.; Ferretti, E.; Borgato, S.; Faglia, G.; Beck-Peccoz, P. Circulating thyrotropin bioactivity in sporadic central hypothyroidism. *J. Clin. Endocrinol. Metab.* **2000**, *85*, 3631–3635. [CrossRef] [PubMed]
26. Grunenwald, S.; Caron, P. Central hypothyroidism in adults: Better understanding for better care. *Pituitary* **2014**, *18*, 169–175. [CrossRef]
27. Persani, L. Clinical review: Central hypothyroidism: Pathogenic, diagnostic, and therapeutic challenges. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 3068–3078. [CrossRef]
28. Adachi, M.; Soneda, A.; Asakura, Y.; Muroya, K.; Yamagami, Y.; Hirahara, F. Mass screening of newborns for congenital hypothyroidism of central origin by free thyroxine measurement of blood samples on filter paper. *Eur. J. Endocrinol.* **2012**, *166*, 829–838. [CrossRef]
29. Kempers, M.J.E.; Lanting, C.I.; van Heijst, A.F.J.; van Trostenburg, S.P.; Wiedijk, B.M.; de Vijlder, J.J.M.; Vulsma, T. Neonatal screening for congenital hypothyroidism based on thyroxine, thyrotropin, and thyroxine-binding globulin measurement: Potentials and pitfalls. *J. Clin. Endocrinol. Metab.* **2006**, *91*, 3370–3376. [CrossRef]
30. Lauffer, P.; Zwaveling-Soonawala, N.; Naafs, J.C.; Boelen, A.; van Trotsenbourg, P. Diagnosis and management of central congenital hypothyroidism. *Front. Endocrinol.* **2021**, *12*, 686317. [CrossRef]
31. Naafs, J.C.; Marchal, J.P.; Fliers, E.; Verkerk, P.H.; Luijten, M.A.J.; Boelen, A.; van Trotsenburg, A.S.P.; Zwaveling-Soonawala, N. Cognitive and Motor Outcome in Patients with Early-Detected Central Congenital Hypothyroidism Compared with Siblings. *J. Clin. Endocrinol. Metab.* **2021**, *106*, e1231–e1239. [CrossRef]
32. Lanting, C.I.; van Tijn, D.A.; Loeber, J.G.; Vulsma, T.; de Vijlder, J.J.; Verkerk, P.H. Clinical effectiveness and cost-effectiveness of the use of the thyroxine/thyroxine-binding globulin ratio to detect congenital hypothyroidism of thyroidal and central origin in a neonatal screening program. *Pediatrics* **2005**, *116*, 168–173. [CrossRef]
33. Naafs, J.C.; Verkerk, P.H.; Fliers, E.; van Trotsenburg, A.S.P.; Zwaveling-Soonawala, N. Clinical and genetic characteristics of Dutch children with central congenital hypothyroidism, early detected by neonatal screening. *Eur. J. Endocrinol.* **2020**, *183*, 627–636. [CrossRef]
34. Boelen, A.; Zwaveling-Soonawala, N.; Heijboer, A.C.; van Trotsenburg, A.S.P. Neonatal screening for primary and central congenital hypothyroidism: Is it time to go Dutch? *Eur. Thyroid. J.* **2023**, *12*, e230041. [CrossRef]
35. Stroek, K.; Heijboer, A.C.; van Veen-Sijne, M.; Bosch, A.M.; van der Ploeg, C.P.B.; Zwaveling-Soonawala, N.; de Jonge, R.; van Trotsenburg, A.S.P.; Boelen, A. Improving the Dutch Newborn screening for central congenital hypothyroidism by using 95% reference intervals for thyroxine-binding globulin. *Eur. Thyroid. J.* **2021**, *10*, 222–229. [CrossRef]
36. Stroek, K.; Heijboer, A.C.; Bouva, M.J.; van der Ploeg, C.P.B.; Heijnen, M.A.; Weijman, G.; Bosch, A.M.; de Jonge, R.; Schielen, P.C.J.I.; van Trotsenburg, A.S.P.; et al. Critical evaluation of the newborn screening for congenital hypothyroidism in the Netherlands. *Eur. J. Endocrinol.* **2020**, *183*, 265–273. [CrossRef] [PubMed]
37. Groeneweg, S.; van Geest, F.S.; Abaci, A.; Alcantud, A.; Ambegaonkar, G.P.; Armour, C.M.; Bakhtiani, P.; Barca, D.; Bertini, E.S.; van Beynum, I.M.; et al. Disease characteristics of MCT8 deficiency: An international, retrospective, multicentre cohort study. *Lancet Diab. Endocrinol.* **2020**, *8*, 594–605. [CrossRef] [PubMed]
38. Friesema, E.C.; Grueters, A.; Biebermann, H.; Krude, H.; von Moers, A.; Reeser, M.; Barrett, T.G.; E Mancilla, E.; Svensson, J.; Kester, M.H.; et al. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* **2004**, *364*, 1435–1437. [CrossRef] [PubMed]
39. Dumitrescu, A.M.; Liao, X.H.; Best, T.B.; Brockmann, K.; Refetoff, S. A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *Am. J. Hum. Genet.* **2004**, *74*, 168–175. [CrossRef]
40. Vatine, G.D.; Al-Ahmad, A.; Barriga, B.K.; Svendsen, S.; Salim, A.; Garcia, L.; Garcia, V.J.; Ho, R.; Yucer, N.; Qian, T.; et al. Modeling Psychomotor Retardation using iPSCs from MCT8-Deficient Patients Indicates a Prominent Role for the Blood-Brain Barrier. *Cell Stem Cell* **2017**, *20*, 831–843.e5. [CrossRef]
41. Iwayama, H.; Kakita, H.; Iwasa, M.; Adachi, S.; Takano, K.; Kikuchi, M.; Fujisawa, Y.; Osaka, H.; Yamada, Y.; Okumura, A.; et al. Measurement of Reverse Triiodothyronine Level and the Triiodothyronine to Reverse Triiodothyronine Ratio in Dried Blood Spot Samples at Birth May Facilitate Early Detection of Monocarboxylate Transporter 8 Deficiency. *Thyroid* **2021**, *31*, 1316–1321. [CrossRef]

42. Groeneweg, S.; Peeters, R.P.; Moran, C.; Stoupa, A.; Auriol, F.; Tonduti, D.; Dica, A.; Paone, L.; Rozenkova, K.; Malikova, J.; et al. Effectiveness and safety of the tri-iodothyronine analogue Triac in children and adults with MCT8 deficiency: An international, single-arm, open-label, phase 2 trial. *Lancet Diab. Endocrinol.* **2019**, *7*, 695–706. [CrossRef]
43. Refetoff, S.; Pappa, T.; Williams, M.K.; Matheus, M.G.; Liao, X.H.; Hansen, K.; Nicol, L.; Pierce, M.; Blasco, P.A.; Jensen, M.W.; et al. Prenatal Treatment of Thyroid Hormone Cell Membrane Transport Defect Caused by *MCT8* Gene Mutation. *Thyroid* **2021**, *31*, 713–720. [CrossRef]
44. Refetoff, S.; Bassett, J.H.D.; Beck-Peccoz, P.; Bernal, J.; Brent, G.; Chatterjee, K.; De Groot, L.J.; Dumitrescu, A.M.; Jameson, J.L.; Kopp, P.A.; et al. Classification and Proposed Nomenclature for Inherited Defects of Thyroid Hormone Action, Cell Transport, and Metabolism. *Eur. Thyroid. J.* **2014**, *3*, 7–9. [CrossRef]
45. Persani, L.; Campi, I. Syndromes of Resistance to Thyroid Hormone Action. *Exp. Suppl.* **2019**, *111*, 55–84. [PubMed]
46. Hauser, P.; Zametkin, A.J.; Martinez, P.; Vitiello, B.; Matochik, J.A.; Mixson, A.J.; Weintraub, B.D. Attention deficit-hyperactivity disorder in people with generalized resistance to thyroid hormone. *N. Eng. J. Med.* **1993**, *328*, 997–1001. [CrossRef] [PubMed]
47. Uter, J.; Heldmann, M.; Rogge, B.; Obst, M.; Steinhardt, J.; Brabant, G.; Moran, C.; Chatterjee, K.; Munte, T.F. Patients with mutations of the Thyroid hormone beta-receptor show an ADHD-like phenotype for performance monitoring: An electrophysiological study. *NeuroImage Clin.* **2020**, *26*, 102250. [CrossRef]
48. Mixson, A.J.; Parrilla, R.; Ransom, S.C.; Wiggs, E.A.; McClaskey, J.H.; Hauser, P.; Weintraub, B.D. Correlations of language abnormalities with localization of mutations in the beta-thyroid hormone receptor in 13 kindreds with generalized resistance to thyroid hormone: Identification of four new mutations. *J. Clin. Endocrinol. Metab.* **1992**, *75*, 1039–1045.
49. Stein, M.A.; Weiss, R.E.; Refetoff, S. Neurocognitive characteristics of individuals with resistance to thyroid hormone: Comparisons with individuals with attention-deficit hyperactivity disorder. *J. Dev. Behav. Pediat* **1995**, *16*, 406–411. [CrossRef]
50. Ferrara, A.M.; Onigata, K.; Ercan, O.; Woodhead, H.; Weiss, R.E.; Refetoff, S. Homozygous thyroid hormone receptor beta-gene mutations in resistance to thyroid hormone: Three new cases and review of the literature. *J. Clin. Endocrinol. Metab.* **2012**, *97*, 1328–1336. [CrossRef]
51. Refetoff, S.; DeWind, L.T.; DeGroot, L.J. Familial syndrome combining deaf-mutism, stuppled epiphyses, goiter and abnormally high PBI: Possible target organ refractoriness to thyroid hormone. *J. Clin. Endocrinol. Metab.* **1967**, *27*, 279–294. [CrossRef]
52. Okosieme, O.E.; Usman, D.; Taylor, P.N.; Dayan, C.M.; Lyons, G.; Moran, C.; Chatterjee, K.; Rees, D.A. Cardiovascular morbidity and mortality in patients in Wales, UK with resistance to thyroid hormone beta (RTHbeta): A linked-record cohort study. *Lancet Diab. Endocrinol.* **2023**, *11*, 657–666. [CrossRef]
53. Campi, I.; Censi, S.; Prodam, F.; Petrone, L.; Brigante, G.; Porcelli, T.; Ruggeri, R.M.; Vigone, M.C.; Rurale, G.; Lio, S.; et al. Increased cardiovascular morbidity and reduced life expectancy in a large Italian cohort of patients with resistance to thyroid hormone β (RTHβ). *Eur. J. Endocrinol.* **2024**, *191*, 407–415. [CrossRef]
54. Radetti, G.; Persani, L.; Molinaro, G.; Mannavola, D.; Cortelazzi, D.; Chatterjee, V.K.; Beck-Peccoz, P. Clinical and hormonal outcome after two years of triiodothyroacetic acid treatment in a child with thyroid hormone resistance. *Thyroid* **1997**, *7*, 775–778. [CrossRef]
55. Takeda, T.; Suzuki, S.; Liu, R.T.; DeGroot, L.J. Triiodothyroacetic acid has unique potential for therapy of resistance to thyroid hormone. *J. Clin. Endocrinol. Metab.* **1995**, *80*, 2033–2040. [PubMed]
56. Groeneweg, S.; Peeters, R.P.; Visser, T.J.; Visser, W.E. Therapeutic applications of thyroid hormone analogues in resistance to thyroid hormone (RTH) syndromes. *Mol. Cel. Endocrinol.* **2017**, *458*, 82–90. [CrossRef] [PubMed]
57. Torre, P.; Bertoli, M.; Di Giovanni, S.; Scommegna, S.; Conte, C.; Novelli, G.; Cianfarani, S. Endocrine and neuropsychological assessment in a child with a novel mutation of thyroid hormone receptor: Response to 12-month triiodothyroacetic acid (TRIAC) therapy. *J. Endocrinol. Investig.* **2005**, *28*, 657–662. [CrossRef] [PubMed]
58. LaFranchi, S.H.; Snyder, D.B.; Sesser, D.E.; Skeels, M.R.; Singh, N.; Brent, G.A.; Nelson, J.C. Follow-up of newborns with elevated screening T4 concentrations. *J. Pediatr.* **2003**, *143*, 296–301. [CrossRef]
59. Vela, A.; Perez-Nanclares, G.; Rios, I.; Rica, I.; Portillo, N.; Castano, L. Spanish Group for the Study of RTH. Thyroid hormone resistance from newborns to adults: A Spanish experience. *J. Endocrinol. Investig.* **2019**, *42*, 941–949. [CrossRef]
60. Bochukova, E.; Schoenmakers, N.; Agostini, M.; Schoenmakers, E.; Rajanayagam, O.; Keogh, J.M.; Henning, E.; Reinemund, J.; Gevers, E.; Sarri, M.; et al. A mutation in the thyroid hormone receptor alpha gene. *N. Engl. J. Med.* **2012**, *366*, 243–249. [CrossRef]
61. van Mullem, A.; van Heerebeek, R.; Chrysis, D.; Visser, E.; Medici, M.; Andrikoula, M.; Tsatsoulis, A.; Peeters, R.; Visser, T.J. Clinical phenotype and mutant TRα1. *N. Engl. J. Med.* **2012**, *366*, 1451–1453. [CrossRef]
62. van Mullem, A.A.; Chrysis, D.; Eythimiadou, A.; Chroni, E.; Tsatsoulis, A.; de Rijke, Y.B.; Visser, W.E.; Visser, T.J.; Peeters, R.P. Clinical phenotype of a new type of thyroid hormone resistance caused by a mutation of the TRα1 receptor: Consequences of LT4 treatment. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 3029–3038. [CrossRef]

63. Van Gucht, A.L.M.; Meima, M.E.; Zwaveling-Soonawala, N.; Visser, W.E.; Fliers, E.; Wennink, J.M.B.; Henny, C.; Visser, T.J.; Peeters, R.P.; van Trotsenburg, A.S.P. Resistance to Thyroid Hormone Alpha in an 18-month-old Girl: Clinical, Therapeutic and Molecular Characteristics. *Thyroid* **2016**, *26*, 338–346. [CrossRef]
64. Furman, A.E.; Dumitrescu, A.M.; Refetoff, S.; Weiss, R.E. Early diagnosis and treatment of an infant with a novel thyroid hormone receptor alpha gene (cC380SfsX9) mutation. *Thyroid* **2021**, *31*, 1003–1005. [CrossRef]
65. Erbas, M.; Çakir, M.D.; Yener, A.S.; Demir, K. Long-term follow-up results and treatment outcomes of children and adults with resistance to thyroid hormone alpha. *J. Endocrinol. Investig.* **2023**, *46*, 1855–1863. [CrossRef] [PubMed]
66. Musolino, A.; Grifoni, G.F.A.; Rodolfi, S.; Andreasi, M.; Bulgarelli, I.; Fanelli, F.; Morelli, V.; Persani, L.; Campi, I. Variable diagnoses in cases referred for discordant thyroid function tests: Focus on lymphoproliferative disorders. *Thyroid* 2025, *in press*.
67. Westbye, A.B.; Aas, F.E.; Kelp, O.; Dahll, L.K.; Thorsby, P.M. Analysis of free, unbound thyroid hormones by liquid chromatography-tandem mass spectrometry: Amini-review of the medical rationale and analytical methods. *Anal. Sci. Adv.* **2023**, *4*, 244–254. [CrossRef] [PubMed]
68. Gelb, M.H.; Basheeruddin, K.; Burlina, A.; Chen, H.J.; Chien, Y.H.; Dizikes, G.; Dorley, C.; Giugliani, R.; Hietala, A.; Hong, X.; et al. Liquid Chromatography–Tandem Mass Spectrometry in Newborn Screening Laboratories. *Int. J. Neonatal Screen.* **2022**, *8*, 62. [CrossRef]
69. Wilson, J.M.G.; Jungner, G. *Principles and Practice of Screening for Disease*; WHO: Geneva, Switzerland, 1968; Available online: https://iris.who.int/handle/10665/37650 (accessed on 18 August 2025).
70. EURORDIS-Rare Diseases Europe. Key Principles for Newborn Screening. A EURORDIS Position Paper. 2021. Available online: https://www.eurordis.org/publications/key-principles-for-newborn-screening/ (accessed on 18 August 2025).
71. Millington, D.S.; Kodo, N.; Norwood, D.L.; Roe, C.R. Tandem Mass Spectrometry: A New Method for Acylcarnitine Profiling with Potential for Neonatal Screening for Inborn Errors of Metabolism. *J. Inher Metab. Dis.* **1990**, *13*, 321–324. [CrossRef]
72. Rashed, M.S.; Ozand, P.T.; Harrison, M.E.; Watkins, P.J.F.; Evans, S.; Baillie, T.A. Electrospray Tandem Mass Spectrometry in the Diagnosis of Organic Acidemias. *Rapid Commun. Mass. Spectrom.* **1994**, *8*, 129–133. [CrossRef]
73. Schulze, A.; Lindner, M.; Kohlmü, D.; Olgemö, K.; Mayatepek, E.; Hoffmann, G.F. Expanded Newborn Screening for Inborn Errors of Metabolism by Electrospray Ionization-Tandem Mass Spectrometry: Results, Outcome, and Implications. *Pediatrics* **2003**, *111*, 1399–1406. [CrossRef]
74. Ruoppolo, M.; Malvagia, S.; Boenzi, S.; Carducci, C.; Dionisi-Vici, C.; Teofoli, F.; Burlina, A.; Angeloni, A.; Aronica, T.; Bordugo, A.; et al. Expanded Newborn Screening in Italy Using Tandem Mass Spectrometry: Two Years of National Experience. *Int. J. Neonatal Screen.* **2022**, *8*, 47. [CrossRef]
75. Oliver-Petit, I.; Edouard, T.; Jacques, V.; Bournez, M.; Cartault, A.; Grunenwald, S.; Savagner, F. Next-Generation Sequencing analysis reveals frequent familial origin and oligogenism in congenital hypothyroidism with dyshormonogenesis. *Front. Endocrinol.* **2021**, *12*, 657913. [CrossRef]

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