Clinical and genetic characteristics of Dutch children with central congenital hypothyroidism, early detected by neonatal screening

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Abstract

Objective: To evaluate clinical characteristics of patients with central congenital hypothyroidism (CH), detected in the Dutch neonatal screening program. This included patients with isolated central CH but the majority have multiple pituitary hormone deficiencies (MPHD).

Design: Nationwide, cross-sectional study.

Methods: Data was collected on clinical characteristics, endocrine tests and neuroimaging of central CH patients, detected by the Dutch neonatal screening and born between 1 January 1995 and 1 January 2015. Height and pubertal status were assessed during a study visit. Isolated central CH patients without a confirmed genetic diagnosis were offered genetic (re-)testing.

Results: During the 20-year period 154 central CH patients were detected (incidence of permanent central CH 1:25 642). After excluding deceased (15), severe syndromic (7) and transient patients (6), 92 of 126 eligible patients were included (57 MPHD; 79% male). Sixty-one patients (50 MPHD) had been hospitalized before screening results were reported, but central CH was diagnosed on clinical grounds in only three of them (5%). MRI abnormalities consistent with pituitary stalk interruption syndrome were seen in 50 (93%) MPHD patients. Among isolated central CH patients, 27 (84%) had an *IGSF1*, *TBL1X* or *IRS4* gene variant (53, 16 and 16%, respectively).

Conclusion: Many patients with central CH have neonatal health problems, especially MPHD patients. Despite hospital admission of two-thirds of patients, almost none were diagnosed clinically, but only after the notification of an abnormal screening result was received. This indicates that central CH, especially if isolated, is an easily missed clinical diagnosis.

European Journal of
Endocrinology
(2020) **183**, 627–636

Introduction

Central congenital hypothyroidism (CH) is characterized by thyroid hormone deficiency at birth due to insufficient hypothalamic-pituitary stimulation of a normal thyroid gland (1, 2). Central CH may occur in isolation, but is

often accompanied by multiple pituitary hormone deficiencies (MPHD) (3, 4).

As CH is harmful to brain growth and development, neonatal screening for CH has been implemented

worldwide since the 1970s. These screening programs are effective in early detection and treatment of CH and, with that, in the prevention of neurodevelopmental disabilities.

Because primary CH, i.e. CH of thyroidal origin, is more prevalent than central CH, most screening programs focus on detecting primary CH, and only measure thyrotropin (TSH) (5). Very few countries employ a thyroxine (T4) or free T4-based screening that also detects central CH (6, 7). Whether detecting central CH should be a screening goal remains a topic of debate. However, it is recognized that early detection of central CH also enables early diagnosis of MPHD, which are potentially life-threatening in case of adrenocorticotrophic hormone (ACTH) deficiency and growth hormone deficiency (GHD), primarily because of severe hypoglycemia (5).

While characteristics of primary CH patients have been described extensively, studies in central CH patients are scarce (4, 8). Clinical characteristics of MPHD patients can be retrieved in part from studies on pituitary stalk interruption syndrome (PSIS) (9). However, not all central CH patients with MPHD have PSIS. Consequently, these patients and patients with isolated central CH are not included in PSIS studies.

Neonatal screening in the Netherlands detects both primary and central CH, and consists of primary T4 measurement, followed by TSH measurement in the lowest 20% of T4 concentrations. To reduce the number of false-positives due to T4-binding globulin (TBG) deficiency, TBG measurement in the lowest 5% of T4 concentrations was added in 1995 (7). This enables calculation of the T4/TBG ratio, providing an estimation of the FT4 concentration. Evaluation studies show the Dutch three-step approach effectively detects primary and central CH, resulting in the highest prevalence of central CH worldwide (1 in 16 404) (7).

Although guidelines acknowledge the value of neonatal central CH screening, the vast majority of neonatal screening programs detect only primary CH (5, 10). Yet, a pilot study for a neonatal screening program aiming at central CH detection was performed recently in Argentina, and the risk of not detecting central CH early has gained attention (11, 12). In balancing the pros and cons of central CH screening, the clinical characteristics of early-detected patients are an important consideration. We performed a cross-sectional study in central CH patients identified by the Dutch neonatal screening over a 20-year period; our objective was to report on the clinical, endocrine, neuroradiological and genetic characteristics of these patients.

Subjects and methods

Patient recruitment

Children with an abnormal neonatal CH screening result are registered in a national database founded by the Netherlands Organization for Applied Scientific Research (TNO). Permission to use this database was obtained from the Privacy Committee of the Dutch CH Screening Board. All central CH patients born between 1 January 1995 and 1 January 2015 and detected through neonatal screening were invited to participate in the study (Supplementary File 1, see section on [supplementary materials](#) given at the end of this article). The study protocol was approved by the local medical ethics committee of Amsterdam UMC, location AMC. All participants gave informed consent for medical chart review and, if applicable, physical examination during the study visit. Written permission was obtained from patients ≥ 12 years and from both parents for patients younger than 18 years.

Data collection

Medical charts were reviewed for data on the medical history, endocrine function testing, neuroimaging and genetic analysis. A structured interview was conducted with one or both parents, and a physical examination was performed. Standard deviation scores (SDS) for current height were calculated using the Dutch reference data from the Growth Analyzer VE (Rotterdam, the Netherlands). SDS for birth weight and length were calculated with the Niklasson reference standards (13). To assess growth, we compared current height-SDS with target height (TH)-SDS for all isolated central CH patients, and GHD patients who had been treated with GH for at least three years.

To confirm the diagnosis of central CH, FT4 concentrations before treatment and results from thyrotropin-releasing hormone (TRH) stimulation tests were reviewed using the local hospital's reference intervals. During TRH testing, TSH concentrations are measured at fixed time points after intravenous TRH administration (standard dosage is 10 μ g/kg, max 200 μ g). Healthy infants show a type 0 response, with a TSH peak >15 mIU/L in the first 30 min, returning to baseline within 3 hours (14). A type 2 response is characterized by deficient TSH increase, and is often seen in patients with isolated central CH; a type 3 response, i.e., slightly delayed but excessive TSH increase followed by a delayed decrease, is frequently seen in MPHD patients (14).

The assessment of additional pituitary hormone deficiencies and MRI studies are described in Supplementary File 1.

Genetic analysis

Isolated central CH patients without a prior confirmed genetic diagnosis were offered genetic (re-)testing with a targeted gene panel, including the following genes: *ANOS1*, *BMP4*, *CHD7*, *FGF8*, *FGFR1*, *GLI2*, *HESX1*, *IGSF1*, *IRS4*, *LHX3*, *LHX4*, *OTX2*, *PAX6*, *POU1F1*, *PROK2*, *PROKR2*, *PROP1*, *SHH*, *SOX2*, *SOX3*, *TBL1X*, *TRH*, *TRHR* and *TSHB*, using Next Generation Sequencing (NGS). NGS was performed with MiSeq, using MiSeq v2 reagents; 2 × 150 bp reads (Illumina, Inc., San Diego, USA). Nimblegen SeqCap EZ Choice (Roche Diagnostics) was used for target enrichment. Since a genetic diagnosis is only established in 5–10% of MPHD patients, and mostly in patients with syndromic forms, genetic (re-)testing was not performed in MPHD patients (15). However, in some patients, genetic analysis had been previously performed by the treating pediatrician ($n=18$); these results are also reported.

Statistical analysis

Data are reported as mean $\pm$ s.d. or median and range, depending on the distribution of data. Comparisons between MPHD patients and isolated central CH patients

were made using t -tests for normally distributed data and Mann–Whitney U tests for data that were not-normally distributed. Categorical characteristics were compared using the Chi-squared test or Fisher's exact test. In current guidelines, CH severity is classified based on pre-treatment plasma FT4 concentrations, but neonatal FT4 concentrations are known to differ from day to day (10, 16). Multiple linear regression was therefore used to compare pre-treatment FT4 concentrations between patient groups, including age in days to correct for the day of measurement. Differences were considered significant in case of two-sided P -values < 0.05 . Analyses were performed with RStudio version 3.6.1 (2019-07-05); graphs were designed using ggplot2 (17, 18).

Results

During the study period, 3 813 329 children were born of whom 7705 (0.2%) died before the neonatal screening could be performed. Of the remaining children, 99.7% participated in the neonatal screening. Central CH was diagnosed in 154 children, of whom 94 (61%) with MPHD and 60 (39%) with isolated central CH (Fig. 1). Permanent central CH was diagnosed in 148 patients, resulting in a calculated incidence of 1:25 642. Six cases were transient (five isolated central CH, one MPHD). Of these, three isolated cases were due to maternal Graves

Figure 1

Patients with central CH identified by the Dutch neonatal screening within a 20-year period. CDG, congenital disorder of glycosylation; CH, congenital hypothyroidism; KAT6A, K acetyltransferase 6A; MEB, muscle-eye-brain disease; MPHD, multiple pituitary hormone deficiencies; SOD, septo-optic dysplasia. †, deceased patient.

disease, which is a well-known cause of transient isolated central CH (19). The remaining three patients could discontinue replacement therapy in childhood; no cause was identified.

Fifteen patients had died and have been reported previously (3). In summary, the high mortality rate could be explained by congenital malformations/syndromes, early infection and birth asphyxia. In only one MPHD patient the cause of death was attributed to pituitary insufficiency (Addisonian crisis).

After excluding deceased patients ($n=15$), severe syndromic patients ($n=7$) and patients with transient central CH ($n=6$), 126 eligible patients remained. Ninety-two patients (57 MPHD), originating from 89 families, gave permission for medical chart review. From three families, two siblings were included; all had a genetic form of isolated central CH. No syndromes were present among included patients, except for two patients with mild septo-optic dysplasia. In one of these patients, the diagnosis septo-optic dysplasia was already made prenatally, with a hospital admission directly after birth. The other patient was admitted for sepsis-like illness on day 4, but the diagnosis was made only after screening results suggestive for central CH and subsequent endocrine evaluation. Both patients suffer from severely impaired vision in at least one eye.

Early signs and symptoms

Thirty-six MPHD patients (63%) and 30 isolated central CH patients (86%) were born after an uneventful pregnancy, i.e., without any reported maternal health problems or medication. For the remaining mothers, health problems were mainly pregnancy-related, for example, pregnancy-induced hypertension and gestational diabetes. An overview of perinatal and clinical characteristics is presented in Table 1.

Hypoglycemia, hyperbilirubinemia and sepsis-like illness occurred more frequently in MPHD patients, with lower glucose concentrations during hypoglycemia in MPHD patients than in isolated central CH patients (mean difference -0.9 mmol/L, 95% CI -0.3 to -1.5 mmol/L, $P < 0.001$). For hyperbilirubinemia, phototherapy sufficed in all patients. Three MPHD patients had neonatal cholestasis which resolved with adequate hormone replacement therapy (consisting of levothyroxine in one patient, and the combination of levothyroxine and hydrocortisone in the other two patients).

Sixty-one patients were hospitalized before screening results were known. During these admissions, central CH

was diagnosed in only three patients (5%). In the other 95%, the diagnosis was made only after screening results were reported. In 79 patients (86%) treatment was started in the first month of life.

Biochemical characteristics

Pre-treatment FT4 concentrations were significantly lower in MPHD patients than in isolated central CH patients (mean difference -1.7 pmol/L, 95% CI -2.7 to -0.7 , $P < 0.001$). TRH testing was performed in 65 patients. Twenty-six patients had a type 2 response; 81% of them had isolated central CH. Thirty-seven patients had a type 3 response; 95% of them had MPHD. A type 0 response (physiological) was seen in one isolated central CH patient. In one patient the test failed due to technical reasons. TRH test results ($n=60$) are shown in Fig. 2.

Other pituitary hormone deficiencies and growth

The frequency of pituitary hormone deficiencies other than TSH is shown in Table 1. Various combinations of pituitary hormone deficiencies were seen among MPHD patients: TSH+ACTH ($n=2$; 4%); TSH+GH ($n=8$; 14%); TSH+ACTH+GH ($n=27$; 47%), and TSH+ACTH+GH+LH/FSH ($n=20$; 35%). Twenty-nine MPHD patients were too young to determine the presence of gonadotropin deficiency (GD). Assessment and outcome of additional pituitary hormone deficiencies in MPHD patients are described in Supplementary File 1.

To assess growth, the difference between current height-SDS and TH-SDS was calculated for all isolated central CH patients with an available TH ($n=34$). Of these, 32 patients (94%) had a current height within TH range. Mean difference between current height-SDS and TSH-SDS was -0.3 ± 1.0 s.d.

Among GHD patients receiving GH for at least 3 years, TH was available for 48 patients (median duration of GH treatment 10.5 years). Mean difference between current height-SDS and TH-SDS was -0.11 ± 0.9 in these patients; 47 (98%) had a current height within TH range.

Imaging

Brain MRI results from 73 patients were available. In 23 cases (19 isolated central CH), no abnormalities were found. MRI results of MPHD patients ($n=54$), including the various combinations of pituitary abnormalities, are shown in Fig. 3.

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Table 1 Clinical characteristics of 92 patients with central CH born between 1 January 1995 and 1 January 2015. Data are presented as n (%), median (range) or mean ($\pm$ S.D.). Dried blood spot (DBS) concentrations are derived from the first neonatal screening assessment.

	Central CH as part of MPHD (n = 57)	Isolated central CH (n = 35)	P-value
Demographic data			
Age at follow-up (years)	10.1 (3–22)	13.0 (4–24)	0.51
Male (%)	41 (72%)	32 (91%)	0.05
Perinatal data			
Gestational age	39 ^{2/7} (34 ^{1/7} –42 ^{3/7})*	40 ^{5/7} (34 ^{2/7} –42 ^{2/7})	0.005
Birthweight (g)	3199 $\pm$ 594*	3684 $\pm$ 655	<0.001
Birthweight, s.d.	–0.4 $\pm$ 1.1*	0.3 $\pm$ 1.3	0.02
Breech position at delivery	17 (30%)	0 (0%)	<0.001
Cesarean section	14 (25%)	4 (11%)	0.20
Apgar < 6 at 5 min	5 (9%)	0 (0%)	0.15
Birth trauma	11 (19%)	0 (0%)	0.006
Clinical features			
Micropenis in males	18 (45%)	0 (0%)	<0.001
Cryptorchidism in males	2 (5%)	1 (3%)	1
Umbilical hernia	4 (7%)	3 (9%)	1
Neonatal health problems			
Hypoglycemia	30 (55%) [†]	5 (14%)	<0.001
Glucose during hypoglycemia (mmol/L)	1.2 $\pm$ 0.8 [¶]	2.1 $\pm$ 0.5	0.008
Feeding problems	33 (60%) [†]	13 (37%)	0.06
Hypothermia	22 (39%)	8 (23%)	0.18
Hyperbilirubinemia	30 (53%)	5 (14%)	<0.001
Sepsis-like illness	24 (42%)	6 (17%)	0.02
Hospitalized prior to abnormal neonatal screening result	50 (88%)	11 (31%)	<0.001
Discharged without a diagnosis of central CH	28 (49%)	7 (20%)	0.01
Diagnosis of (central) CH prior to abnormal neonatal screening result	3 (5%)	0 (0%)	0.29
Screening results and age at diagnosis			
DBS T4 concentration (nmol/L)	54 (8–153)	60 (20–145)	0.79
DBS T4, s.d.	–2.3 (–4.3 to –0.4)	–2.4 (–3.8 to –0.16)	0.35
DBS TSH concentration < 3 mU/L	34 (63%) [‡]	17 (34%) [§]	0.33
DBS T4/TBG ratio (abnormal if <17)	9.0 (2.9–16.1) [‡]	8.0 (4.2–16.8) [§]	0.42
Venous FT4 concentration (pmol/L) at first diagnostic measurement	8.7 $\pm$ 2.1	10.4 $\pm$ 2.4	<0.001
Age at first diagnostic measurement (days)	13 (2–135)	14 (5–58)	0.22
Age at start of treatment (days)	17 (2–135)	21 (7–162)	0.13
Pituitary hormone deficiencies			
Growth hormone deficiency	55 (96%)	1 (3%) [§]	NA
ACTH deficiency	49 (86%)	0 (0%)	NA
Gonadotropin deficiency	20 (74%)	0 (0%)	NA

^aPartial growth hormone deficiency was diagnosed in one patient with *IGSF1* deficiency syndrome. *n = 56; [†]n = 55; [‡]n = 54; [§]n = 34; [¶]n = 28; ^{||}n = 27.

ACTH, adrenocorticotrophic hormone; CH, congenital hypothyroidism; DBS, dried blood spot; MPHD, multiple pituitary hormone deficiencies; NS, neonatal screening; T4, thyroxine; TBG, thyroxine-binding globulin; TSH, thyrotropin.

Genetic analysis

Genetic analysis had already been performed in 22 isolated central CH patients, and was performed in 10 additional patients for this study. The three remaining patients refused genetic testing. In 91% of tested patients, we found variants in genes associated with isolated central CH, including eight novel variants (four *IGSF1*;

three *TBL1X*; one *IRS4*). All identified variants were pathogenic, except for two novel variants (*TBL1X* and *IGSF1*), and one known *IGSF1* variant (Table 2) (20). In silico prediction of the novel variants of unknown significance suggested pathogenicity. The *TBL1X* variant was predicted to cause abnormal protein splicing and possibly a frame-shift mutation, and was de novo (mother did not carry the variant). The *IGSF1* variant

Figure 2

TSH concentrations during TRH tests in children with central CH ($n = 60$). CH, congenital hypothyroidism; MPHD, multiple pituitary hormone deficiencies.

caused an amino acid substitution probably leading to abnormal protein function. The healthy mothers of the two patients with the *IGSF1* variant were not willing to undergo genetic analysis themselves. No male siblings were present.

Among MPHD patients, genetic analysis had been performed prior to our study in 18 patients. The targeted gene panel for central CH, as used in the current study, was

Figure 3

The incidence of pituitary abnormalities in patients with central congenital hypothyroidism and multiple pituitary hormone deficiencies (54 MRI scans available). Two patients (one complete PSIS; one SOD) also had optic nerve hypoplasia. PSIS, pituitary stalk interruption syndrome; SOD, septo-optic dysplasia.

performed in 10 patients. Abnormalities were found in two patients. One patient was heterozygous for a *POU1F1* variant (c.143T>G, p.Val48Gly); this variant was not present in the patient's healthy parents and was classified as likely pathogenic. In the same patient, an *FGFR3* variant of unknown significance was identified (c.1388A>G, p.Tyr463Cys), which was present in patient's healthy father as well. In the second patient, a *SOX3* variant of unknown significance was identified (c.696C>A, p.(?)). Whether this variant was de novo could not be retrieved from medical charts.

In the remaining eight patients, gene analysis focused on one or several genes associated with hypopituitarism or central CH (*HESX1*: five patients; *PROT1*: two patients; *TBL1X*: two patients; *LHX3*: one patient; *SOX2*: one patient; *IRS4*: one patient). No abnormalities were found in these patients.

Discussion

In this study, we describe the clinical characteristics of 92 patients with central CH, both isolated and as part of MPHD, after early detection in the Dutch neonatal screening program during a 20-year period. This is the largest group of early-detected central CH patients reported on to date, providing insight into the yield of a central CH screening program. The incidence of central CH in the current study (1:25 642) is similar to that previously reported in the Netherlands (1:25 000 for the period 1981–1990) (21). The Dutch central CH incidence rate thus remained stable, but is higher than in previous studies on T4-based screening programs in Buenos Aires and Indiana (4, 11). This might be due to truly lower incidences in these countries. However, an alternative explanation is that these programs use more strict cut-offs: in Buenos Aires a T4 cut-off of 4.5 µg/dL (58 nmol/L; -2.3 s.d.) was used, in Indiana the cut-off was 5 µg/dL (64 nmol/L; -2.0 s.d.). The Dutch screening program does not use a fixed T4 cut-off. Instead, an additional TSH measurement is performed when T4 concentration is <-0.8 s.d. of the daily mean (lowest 20%), and both TSH and TBG are measured when T4 concentration is <-1.6 s.d. (lowest 5%). While newborns with a T4 <-3.0 s.d. are immediately referred, newborns with a T4 between -0.8 and -3.0 s.d. are referred based on TSH and TBG concentrations (22). If TSH is inappropriately low and the T4/TBG ratio does not imply (relative) TBG deficiency, the screening result is considered inconclusive, and a second screening is performed. As a result of this unique program, a higher

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Table 2 Results from genetic analysis in 32 patients with isolated central CH, born in the period 1 January 1995 to 1 January 2015.

Gene	Patients, n (%)	Nucleotide alteration	Amino acid alteration
<i>IGSF1</i>	17 (53%); 15 families	c.1847G>A [†] c.1981G>A (<i>n</i> = 2) [‡] c.2267G>A + c.2278G>T c.2388del [†] c.2248delG (<i>n</i> = 3; 2 families) c.3032delinsTT c.3049C>T (<i>n</i> = 2; 1 family) c.3416G>T ^{†,‡} c.3565C>T c.3691T>G c.3767-1G>A c.(?-1) ₋ (4026+?)del [†] c.328-kb deletion, arr Xq26.1q26.2	p.Trp616* p.Gly661Arg p.Arg756His + Glu760* p.Gly797Valfs*4 p.Glu750Lysfs*28 p.Gly1011Valfs*14 p.Gln1017* p.Cys1139Phe p.Arg1189* p.Cys1231Gly ^a b c
<i>IRS4</i>	5 (16%); 4 families	c.1772dup (<i>n</i> = 2) c.554C>A [†] c.643G>T (<i>n</i> = 2; 1 family)	p.Lys592Glnfs*12 p.Ser185* p.Gly215*
<i>TBL1X</i>	5 (16%)	c.1152del [†] c.1246A>T c.1510C>T c.705G>A ^{†,‡} c.357+1G>A [†]	p.Phe385Leufs*50 p.Asn365Tyr p.His453Tyr p.Val235= p.(?)
<i>TSHB</i>	1 (3%)	c.373delT	p.Cys125Valfs*10
<i>TRH-R</i>	1 (3%)	c.49C>T	p.Arg17*
No genetic cause found	3 (9%)		

[†]Novel variant; [‡]Variant of unknown significance; ^aSplice site mutation which may cause frameshift and premature stop codon; ^bDeletion of the entire coding sequence; ^cDecreased membrane expression and glycosylation as described by Joustra et al. (20).

number of central CH patients is detected, including milder cases with T4 concentrations which are on average higher compared to cases from Buenos Aires and Indiana (Table 1). It also explains why the proportion of isolated central CH patients compared to MPHD is higher than in previous studies. Isolated central CH patients have on average higher pre-treatment FT4 concentrations than MPHD patients, and are thus detected more easily in the Dutch screening program.

A male predominance was seen in the entire group, in line with previous studies (7, 23). For isolated central CH this can be explained by associated X-linked genes *IGSF1*, *IRS4* and *TBL1X* (20, 24, 25). While male predominance has also been described in patients with congenital hypopituitarism or PSIS (55–85%), the explanation is less clear for MPHD patients (9, 26, 27). Variants of X-linked genes in congenital MPHD (*SOX3*; *EIF2S3*) are very rare. Overall, a genetic cause is found in only 5–10% of MPHD patients (15).

Most MPHD patients (88%) were hospitalized before the neonatal screening results became known, for example, for hypoglycemia or sepsis-like illness. Hypoglycemia and sepsis-like illness are known effects of

ACTH deficiency, may be aggravated by concomitant GHD and can be life-threatening if left untreated. Neonatal cholestasis is another known manifestation of MPHD (28). It is attributed to both cortisol deficiency and GHD, as glucocorticoids increase bile flow and GH is involved in the synthesis and excretion of bile acids (28, 29). The role of central CH in neonatal cholestasis in MPHD patients is unclear. The low prevalence of neonatal cholestasis in our study may be related to the early start of hormone treatment, particularly of hydrocortisone.

Despite neonatal hospitalization in 61 patients, central CH was diagnosed purely on clinical grounds in only three of them (5%). Most patients were even discharged, and readmitted because of the abnormal screening result. These results emphasize the importance of central CH screening. To date, central CH is the only pituitary hormone deficiency suitable for a neonatal screening program. Detection through screening led to early diagnosis and treatment of MPHD, and MPHD patients started thyroxine at a median age of 17 days. However, when central CH is not detected by neonatal screening, a mean age at endocrine consultation of 16 months is reported (4). The fact that central CH is missed

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despite early symptoms has been reported in smaller studies before. Mehta et al described 54 central CH patients of whom only 28% were diagnosed during the neonatal period, although 72% had neonatal problems (23). Similar results are seen in PSIS patients (9). These cases, missed despite early symptoms, highlight the importance of neonatal screening for central CH. They also underline the need for improving awareness of this rare disorder among clinicians.

The diagnosis of isolated central CH was genetically confirmed in the majority of patients. *IGSF1* variants were seen in 53%. *IGSF1* deficiency currently is the most common genetic cause of isolated central CH; in a previous Japanese study *IGSF1* variants were identified in 38% of patients (20, 30). The current study suggests an even higher proportion of patients with *IGSF1* deficiency as a cause of isolated central CH.

The value of TRH stimulation testing in diagnosing central CH is controversial (23). Although the majority of central CH patients show an abnormal response, a normal response does not rule out central hypothyroidism. The role of TRH testing in diagnosing central hypothyroidism is therefore considered 'modest' (23, 31). Van Tijn *et al.* performed a study in 15 infants with central CH and observed an abnormal TSH response in all patients (14). They concluded that especially a type 3 response may help distinguishing neonates with central CH from infants with a false-positive screening result. In the current study, a type 3 response was almost exclusively seen in MPHD patients, which emphasizes the value of TRH testing when central CH with MPHD is suspected. Because only central CH patients were included, TRH testing results could not be compared with healthy neonates.

ACTH deficiency was present in 49 (86%) MPHD patients and was diagnosed within the first 6 months of life in 44 (90%) patients. It is important to realize that in patients with pituitary malformations, ACTH deficiency may emerge later in life (32). In one patient ACTH deficiency was diagnosed by performing a low dose ACTH test at the age of 14 years, while a neonatal glucagon stimulation test and early morning serum cortisol measurements throughout childhood were normal. This emphasizes the need to continue monitoring patients, even into adulthood.

The majority of MPHD patients (96%) had GHD. Previous studies show that GHD is present in 100% of PSIS patients, whereas central CH is reported in 48–80% of PSIS patients (9, 33). The high GHD prevalence found in the current study indicates that GHD is almost always present in MPHD patients identified through central

CH screening. GHD was diagnosed in one patient with isolated central CH due to *IGSF1* deficiency syndrome. Transient partial GHD has been described in 14% of *IGSF1* deficiency patients (20).

Among MPHD patients old enough for pubertal assessment, GD was present in 20 patients (74%). This corresponds with previous studies reporting GD in 66–80% of patients with PSIS and MPHD (9, 34). A previous study on congenital GD raised the question whether boys with MPHD and a normal penis could still have GD (35). Five GD patients did not have a history of micropenis or cryptorchidism, implying that normal male genitalia early in life does not exclude GD. In addition, Rottembourg et al reported that two of 11 PSIS patients with GD had a normal penis (34). On the other hand, the presence of micropenis does not always indicate GD; it has also been observed in (isolated) GHD (36). In our study, one MPHD patient with a micropenis at birth had a spontaneous onset of puberty at the age of 11.

Our study has both strengths and limitations. A strength is the inclusion of a large group of early-detected central CH patients, originating from a nationwide sample. The use of a national database and the long follow-up period enabled us to provide a complete overview of patients and their definitive diagnoses. Moreover, we provide detailed clinical data of MPHD and isolated central CH patients. Previous studies in MPHD often focused on specific groups, for example, PSIS patients, while studies on isolated central CH are even more scarce, except for studies regarding the *IGSF1* deficiency syndrome (9, 20).

A limitation of our study is that, as patients were diagnosed and treated in different hospitals, various laboratory assays were used, which is especially important for the neonatal FT4 measurement. In addition, included patients were diagnosed in a 20-year period, during which MRI techniques improved considerably. Finally, although we obtained all data available for 92 patients, no data could be reviewed for 47 patients, and a certain degree of selection bias cannot be excluded. The retrospective nature of most assessments performed in this study can also be considered a limitation.

In conclusion, we provide an overview of the yield of 20 years of neonatal screening for central CH in the Netherlands, which may be valuable for countries considering to adjust their CH screening program to include the detection of central CH. In 91% of isolated central CH patients, the diagnosis was genetically confirmed by identification of *IGSF1*, *TBL1X*, *IRS4*, *TSHB* or *TRH-R* variants. Pituitary abnormalities were not seen in isolated central CH patients, while the majority of

MPHD patients had pituitary abnormalities compatible with PSIS. Most MPHD patients, and one-third of isolated central CH patients, were hospitalized in the neonatal period for severe hypoglycemia and sepsis-like illness. However, despite these hospital admissions almost none of the patients were diagnosed clinically. Instead, central CH was diagnosed only after the neonatal screening result turned out to be abnormal, often even after the patient had already been discharged. This illustrates the importance of a T4-based neonatal screening program to ensure early diagnosis of central CH. It also highlights the need to improve awareness of this rare disorder among clinicians since most patients, especially in case of MPHD, were not recognized despite the presence of classic symptoms such as hypoglycemia, jaundice and micropenis.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-20-0833>.

Declaration of interest

N Z-S has received a grant from Pfizer to support this investigator-initiated study (tracking number WI219179). Pfizer was not involved in patient recruitment, data collection, data analysis or preparation of the manuscript. All other authors declare no competing interests.

Funding

Support for this investigator-initiated study was provided by Pfizer (tracking number WI219179).

Author contribution statement

A S P v T and N Z-S contributed equally to this work. N Z-S, A S P v T, P H V and E F designed the study. J C N acquired data, designed the statistical plan, analyzed and interpreted data, and wrote the manuscript. N Z-S supervised the study, interpreted data, and critically reviewed the manuscript. P H extracted data and revised the manuscript. A S P v T supervised the study, interpreted data, and critically reviewed the manuscript. E F critically reviewed the manuscript. A S P v T and N Z-S had full access to all of the study data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgement

The authors would like to thank Brenda Wiedijk (Amsterdam UMC, location AMC) for her help with patient recruitment and collection of the medical charts.

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Received 20 July 2020

Revised version received 08 September 2020

Accepted 30 September 2020