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Neonatal Detection of Congenital Hypothyroidism of Central Origin

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Due to the high frequency of concurrent pituitary hormone deficiencies, congenital hypothyroidism (CH) of central origin (CH-C) is a life-threatening disorder. Yet only a minority of these patients are detected by neonatal CH screening programs worldwide. We conducted a prospective multicenter study involving a 2-yr cohort of neonatally diagnosed CH-C patients to determine whether a T_4-TSH-based neonatal CH screening protocol extended with T_4 binding globulin determinations improves early detection of CH-C and to assess the extent of pituitary hormone deficiency among the identified CH-C patients. In all infants with screening results indicative of CH-C, the functional integrity of the hypothalamo-hypophyseal system was investigated by dynamic tests; the anatomical integrity was investigated by magnetic resonance imaging. Initial test results were evaluated after 5 yr of follow-up. Among 385,000 infants screened over the 2-yr period, 19 cases of permanent CH-C were detected (prevalence, 1:20,263; 95% confidence interval, 1:12,976 to 1:33,654), representing 13.5% of all detected cases of permanent CH. The majority (78%) had multiple pituitary hormone deficiency, whereas 53% had pituitary malformations on magnetic resonance imaging. We conclude that infants with CH-C can very well be detected by neonatal screening. The estimated prevalence and the severity of pituitary dysfunction of this treatable disorder call for explicit attention for this entity of CH in neonatal screening programs worldwide. **(*J Clin Endocrinol Metab* 90: 3350–3359, 2005)**

CONGENITAL DISORDERS OF the hypothalamo-hypophyseal system have been known since the mid-1950s (1, 2), but comprehension of the pathogenesis of this gamut of disorders was long obscured by misinterpretation of birth characteristics and imaging studies in the pre-magnetic resonance imaging (MRI) era. Until the early 1990s, these disorders were generally considered the result of birth traumata (3). Only after the revelation of the well-defined magnetic resonance image of posterior pituitary ectopia was the plausibility of a developmental disorder recognized (4, 5). During the past decade, various transcription factors and the corresponding genes involved in pituitary development and function have been discovered. Defects in these genes give rise to multiple pituitary hormone deficiency and pituitary hypoplasia (6–11). Two of these genes are associated with posterior pituitary ectopia in a minority of cases (10, 12).

Despite the severity of hormonal dysfunction, congenital disorders of the hypothalamo-hypophyseal system are seldom diagnosed in early infancy. In contrast to a TSH-based screening program, a T_4-based neonatal screening for congenital hypothyroidism (CH) is suited in principle for early detection of cases of CH of central origin (CH-C) (13), thus disorders of the hypothalamo-hypophyseal system. Unawareness of the vital risks and ignorance of the possibilities to identify these patients by neonatal screening (13) have hampered the development of diagnostic protocols apt to detect this life-threatening entity of CH.

In 1995, after a 1-yr trial period, the Dutch neonatal CH-screening program, based on initial T_4 and consecutive TSH determination, was extended with a determination of T_4 binding globulin (TBG) (14). A pilot study had indicated that this addition, besides reduction of the number of false-positive screening results, could improve detection of CH-C (15). Therefore, for a period of 2 yr, all children with screening results indicative of CH-C were enrolled in a nationwide prospective study to determine whether the neonatal CH screening protocol extended with TBG determinations improves early detection of CH-C and to assess the extent of pituitary hormone deficiency among the identified CH-C patients. Of all patients detected by neonatal screening, the integrity of the hypothalamo-hypophyseal system was investigated following a standardized protocol of endocrine function testing and neuroradiologic imaging. We report the major results after 5 yr of follow-up of the cohort of 19 patients with permanent CH-C, detected by neonatal screening.

Patients and Methods

Neonatal CH screening

The neonatal CH screening procedure was based on determination of T_4 in dried blood spots obtained by heel puncture 5–7 d after birth and consecutive TSH determination of those samples in the lowest 20% of T_4 concentrations and TBG of those samples in the lowest 5% of T_4 concentrations (Fig. 1). The ratio between the T_4 and TBG concentrations was used as a measure of the free circulating T_4 concentration. In a pilot study, the cut-off value for the T_4 to TBG ratio was estimated at 8.5 (15). To prevent delayed diagnosis of the most severe cases, children with

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Abbreviations: CH, Congenital hypothyroidism; CH-C, CH of central origin; ICMA, immunochemiluminometric assay; IGFBP-3, IGF binding protein 3; MRI, magnetic resonance imaging; TBG, T_4 binding globulin; T_4-SDS, T_4 SD score.

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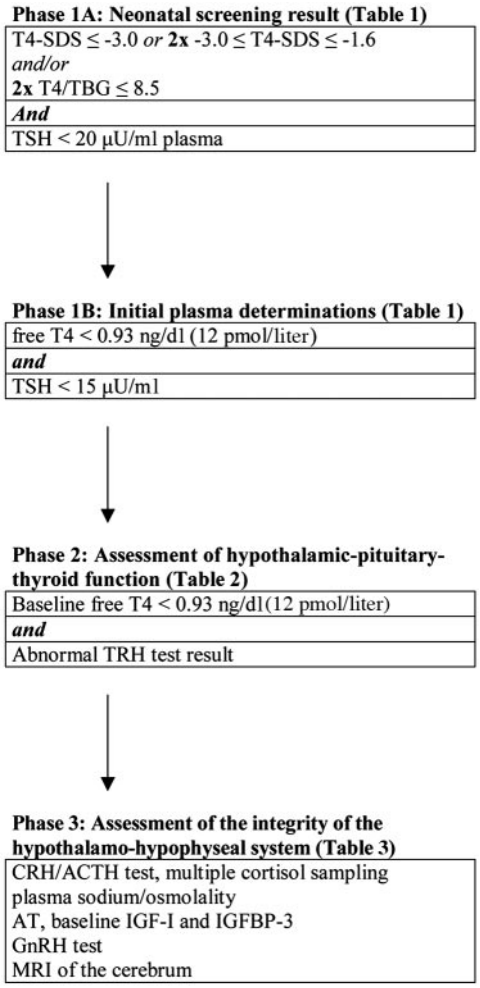

FIG. 1. Study design (flow chart). Neonatal CH screening procedure and inclusion criteria. T_4 was measured in all heel puncture samples. Of those samples in the lowest 20% of T_4 concentrations (*i.e.* ≤0.8 SD below the mean), the TSH concentration was measured in the same sample. Of those samples in the lowest 5% of T_4 concentrations (*i.e.* ≤1.6 SD below the mean), the TBG concentration was measured additionally. T_4/TBG was calculated by the following division: T_4-SDS + 5.1/TBG (nanomoles per liter). In a pilot study, the cut-off value for the T_4 to TBG ratio was estimated at 8.5 (15). To prevent delayed diagnosis of the most severe cases, children with TSH concentrations of at least 50 μU/ml plasma and/or T_4-SDS no greater than −3.0 were directly referred to a pediatrician. Children with borderline TSH (20–50 μU/ml plasma), and/or borderline T_4-SDS (−1.6 to −2.9), and/or T_4 to TBG ratio below 8.5 underwent a second heel puncture. When the result was abnormal or again borderline, the child was referred to a pediatrician. The screening result was considered indicative of CH-C if T_4-SDS and/or T_4 to TBG ratios were abnormal, with an accompanying heel stick TSH below 20 μU/ml plasma. Children with screening results indicative of CH-C were included in the study if the consecutive venous TSH plasma concentration was below 15 μU/ml (15 mU/liter) and free T_4 was below 0.93 ng/dl (12 pmol/liter). Conversion factors: TBG, 1 μg/dl = 18.52 nmol/liter; TSH, 1 μU/ml = 1 mU/liter; free T_4, 1 ng/dl = 12.87 pmol/liter. T4-SDS, T_4-SDS (of the series of that day); TBG, TBG concentration (in nanomoles per liter); T4/TBG, T_4 to TBG ratio; AT, arginine test.

TSH concentrations more than or equal to 50 μU/ml (50 mU/liter) plasma and/or T_4 SD scores (T_4-SDS) less than or equal to −3.0 were directly referred to a pediatrician. Children with borderline TSH [20–50 μU/ml plasma (20–50 mU/liter)], and/or borderline T_4-SDS (−1.6 to −2.9), and/or T_4 to TBG ratio less than 8.5 underwent a second heel puncture. When the result was abnormal or again borderline, the child was referred to a pediatrician. The screening result was considered indicative of CH-C if T_4-SDS and/or T_4 to TBG ratio was abnormal, with accompanying heel stick TSH less than 20 μU/ml (20 mU/liter) plasma.

Study patients

The study design is depicted in Fig. 1. The subjects were participants in a Dutch nationwide prospective study, running from April 1, 1994, to April 1, 1996. Infants with neonatal CH-screening results indicative of CH-C and subsequent plasma free T_4 concentrations below the beforehand estimated cut-off of 0.93 ng/dl (12 pmol/liter) (16) and plasma TSH concentrations less than 15 μU/ml were enrolled. A diagnosis of CH-C was assessed by a TRH test. Anterior pituitary function was assessed primarily by stimulation tests. TRH and CRH tests took place on consecutive days as soon as the alleged CH-C patient was referred; T_4 supplementation was installed immediately after TRH test results had proven CH-C. Arginine and GnRH tests were performed at the age of 3 months, when euthyroid status had been accomplished by T_4 supplementation, using the opportunity provided by the postnatal surge of gonadotropins and sex steroids to evaluate gonadotropin function (17, 18). Also, in most patients, MRI of the cerebrum was performed around the age of 3 months, to assess the anatomical integrity of the hypothalamo-hypophyseal system. In 2001, all cases were reevaluated for revised diagnoses, additional morbidity, growth, and treatment data. The study protocol was approved by the Dutch Pediatric Endocrine Society and by the Medical Ethics Committees of the participating centers. Parental informed consent was obtained in all cases.

Investigation of the thyrotropic hormone axis

Plasma TSH was measured before and 15, 30, 45, 60, 120, and 180 min after iv administration of TRH (10 $\mu g \cdot kg^{-1}$). An adequate TSH response to TRH (type 0) was defined by a peak concentration exceeding 15 μU/ml (19–21) and return to baseline within 3 h (19, 22). In response to TRH, CH-C patients either show diminished (type 2), or slightly delayed but excessive increase and delayed decrease of the TSH plasma concentration (type 3) (19, 20).

Investigation of the ACTH axis

Plasma ACTH and cortisol were measured before and 5, 10, 15, 30, 45, 60, 120, and 150 min after iv administration of CRH (1 $\mu g \cdot kg^{-1}$). An adequate response was defined by an ACTH peak concentration exceeding 80 pg/ml or four times baseline level and a cortisol peak concentration exceeding 18 μg/dl (500 nmol/liter) or 7 μg/dl (200 nmol/liter) over baseline level (23). In selected cases, a short ACTH test was performed (see Table 2). An adequate response to ACTH was defined by a cortisol peak concentration exceeding 18 μg/dl (500 nmol/liter) or 7 μg/dl (200 nmol/liter) over baseline level (24, 25). Of all patients, multiple random plasma cortisol samples were taken. Concentrations exceeding 18 μg/dl (500 nmol/liter) were considered adequate (23).

Investigation of the somatotropic hormone axis

Plasma GH was measured before and 30, 45, 60, 75, 90, 120, and 180 min after iv administration of arginine (500 $mg \cdot kg^{-1}$). A GH concentration exceeding 10 ng/ml (20 mU/liter) was considered an adequate response (26). Function test results were complemented by baseline plasma IGF-I and IGF binding protein 3 (IGFBP-3) determinations (27).

Investigation of the gonadotropic hormone axis

Plasma LH and FSH were measured before and 15, 30, 45, 60, and 120 min after iv administration of GnRH (10 $\mu g \cdot kg^{-1}$). An adequate response was defined by peak concentrations of LH exceeding 3 mU/ml and FSH exceeding 6 mU/ml in girls, 3 mU/ml in boys (28).

Investigation of posterior pituitary function

In all patients, plasma sodium and potassium concentrations and diuresis rate were determined.

TABLE 1. Phase 1—Perinatal data and neonatal screening results

Subject	Perinatal data						First neonatal screening result					Second neonatal screening result					Inclusion	
	Sex	GA (wk)	BBM [g (percentile)]	Birth mode	Perinatal morbidity	First sign	Age (d)	T_4 [μg/dl (SDS)]	TBG (μg/dl)	T_4/TBG	TSH (μU/ml)	Age (d)	T_4 [μg/dl (SDS)]	TBG (μg/dl)	T_4/TBG	TSH (μU/ml)	Age (d)	FT4 (ng/dl)
1	M	36.9	2310 (3–10)	H	1,2	S	5	0.9 (−4.7)	11.8	1.8	<3						7	0.33
2	F	40.9	2795 (3)	B, SC	1	S	5	6.0 (−2.1)	20.6	7.9	<3	16	6.4 (−2.7)	21.5	6.0	<3	25	0.58
3	F	34.1	2045 (25–50)	B	3,4	C(3)	5	7.2 (−2.3)	25.4	6.0	<3						17	0.58
4	M	41	3830 (50–75)	H, VE	3	S	5	7.8 (−1.9)	24.1	7.2	3.8						49	0.61
5	M	40.4	4680 (>97)	H, VE	5	S	9	7.5 (−2.4)	23.1	6.3	5	13	5.9 (−3.3)	17.1	5.7	5	39	0.64
6	M	40.3	4100 (75–90)	B	3,4,6	S	6	6.5 (−2.4)	19.0	7.7	<2	20	6.2 (−2.3)	18.3	8.3	<2	29	0.65
7	M	41	4950 (>97)	H	3	S	5	6.2 (−3.6)	9.9	8.2	5						36	0.65
8	F	39.7	3390 (50)	H	0	S	15	7.3 (−2.7)	14.1	9.2	<3	28	6.5 (−2.1)	13.5	12.0	<3	49	0.65
9	F	38.1	3230 (50–75)	H	3,4,6	S	7	4.4 (−3.9)	16.3	4.0	5						15	0.66
10	M	41.7	4650 (97)	H	3,4	S	5	6.2 (−2.7)	16.4	7.9	5	12	6.1 (−2.6)	22.5	6.0	5	19	0.71
11	M	40	3000 (10)	H	4	S	130	0.6 (>−4.3)	31.8	1.3	<2						137	0.78
12	M	32	1375 (10)	H, SC	4,7,8	C(7)	8	2.7 (−4.0)	17.1	3.5	<2	29	10.0 (−1.8)				50	0.82
13	M	40	3080 (10–25)	B, FE	1,4,7,9	S	6	8.8 (−1.9)	21.9	7.9	5	19	10.5 (−1.6)	32.3	5.9	5	39	0.84
14	M	42	3600 (25–50)	H	0	S	7	4.6 (−3.2)			1.2						47	0.85
15	M	42	3720 (50)	H	0	S	7	10.4 (−2.1)	22.5	7.2	7	14	7.6 (−2.9)	16.1	7.4	4	23	0.86
16	M	37.4	1785 (<3)	B, FE	3,4,8,9	S	6	7.2 (−2.4)	18.8	7.8	5						777	0.87
17	M	42	3400 (10–25)	H	0	S	6	11.3 (−1.6)	23.1	8.2	5	13	7.7 (−2.2)	22.6	6.9	5	56	0.88
18	M	38.3	1845 (<3)	H, FE	3,4,6,7,10	S	8	6.8 (−2.7)	15.5	8.4	<3	15	6.0 (−2.4)	16.4	8.9	<3	26	0.88
19	M	40.7	3530 (25–50)	H	0	S	7	4.4 (−3.3)	9.9	9.8	<3						17	0.91
20	F	42	3220 (10–25)	H	0	S	5	7.0 (−2.7)	16.0	8.1	5	22	6.9 (−3.1)	18.3	5.9	5	36	0.92
21	F	39.7	3000 (10–25)	H	3,11	S	13	4.2 (−3.1)	21.1	5.2	<2						17	0.96
22	M	41.4	4160 (75–90)	H	0	S	7	7.8 (−2.4)	22.4	6.6	8	28	9.3 (−2.3)	22.5	6.3	4	55	0.99
23	M	39.1	3250 (25–50)	H	0	S	8	9.4 (−2.0)	19.8	8.5	3	28	9.6 (−1.8)	22.7	7.8	1.5	40	1.00
24	M	41	3000 (3–10)	H	0	S	6	8.3 (−2.0)	19.8	8.5	5	18	7.9 (−1.9)	21.2	8.1	5	93	1.06
25	M	38.6	2740 (10)	H	12	S	5	1.7 (−3.7)	14.8	5.1	<3	17	9.5 (−1.1)	24.4	8.8	3	47	1.07
26	F	42	3920 (75)	H	0	S	7	3.6 (−3.2)	5.0	20.7	7						14	1.54

Patients were ranked according to plasma free T_4 concentration at inclusion. GA, Gestational age; BBM, birth body mass. Percentiles: Niklasson *et al.* (48). Birth mode: H, head position; B, breech position; SC, cesarean section; VE, vacuum extraction; FE, forcipal extraction. Perinatal morbidity/first sign: 0, no manifest morbidity; 1, asphyxia; 2, cardiorespiratory insufficiency; 3, hypoglycemia; 4, hyperbilirubinemia and/or elevated transaminases; 5, umbilical hernia; 6, persistent vomiting; 7, hypogenitalism; 8, maldescensus testis; 9, lethargy, poor feeding; 10, hyponatremia (transient); 11, neonatal seizures; 12, neonatal sepsis/meningitis; S, congenital hypothyroidism screening result first sign recognized; C, recognition on clinical grounds. Neonatal screening: hormone determinations in eluates of filter paper blood spots, obtained by heel puncture. SDS, SD score. Conversion factors: TSH, 1 μU/ml = 1 mU/liter; T_4, 1 μg/dl = 12.87 nmol/liter; free T_4, 1 ng/dl = 12.87 pmol/liter; TBG, 1 μg/dl = 18.52 nmol/liter.

MRI

To evaluate pituitary morphology, MRI studies were performed under general anesthesia, in most patients using a 1.5 Tesla Siemens Magnetom (Siemens, Munich, Germany). Transversal (5 mm), sagittal (3 mm), and coronal (3 mm) T1-weighted spin-echo images were obtained. In the transversal plane, proton density as well as T2-weighted turbo-spin echo sequences were used also. In addition, a T1-weighted 3D series (MPRage) was taken.

Assays

TSH was measured by immunochemiluminometric assay (ICMA; Behring, Amsterdam, The Netherlands). T_4, T_3, and rT_3 were measured by in-house RIA methods (AMC, Amsterdam, The Netherlands). Free T_4 was measured by a two step RIA assay (SPAC fT4 Fraktion; Byk-Sangtek Diagnostica, Dietzenbach, Germany). TBG was measured by RIA (Eiken Chemical Co., Tokyo, Japan). ACTH was measured by ICMA (Nichols Institute, Wychen, The Netherlands). Cortisol was measured by fluorescence polarization immunoassay (TDx; Abbott, Amstelveen, The Netherlands). LH and FSH were measured by ICMA (Amerlite; Amersham Biosciences, Little Chalfont, UK). 17-β-Estradiol was measured by RIA (estradiol-2; Sorin Biomedica, Saluggia, Italy). Testosterone was measured by RIA (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA). GH was measured by RIA (Spectria/Orion, Turku, Finland). IGF-I and IGFBP-3 were measured by RIA (in-house assays Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands).

Statistical analysis

SPSS 10.1 (SPSS, Inc., Chicago, IL) was used for statistical computations. All reported *P* values are two-sided.

Results

Study patients

Of 385,000 infants screened during the study period, 26 met with the inclusion criteria. Perinatal characteristics and endocrine test results of these subjects are summarized in Tables 1, 2, and 3.

Nineteen infants were diagnosed with permanent CH-C. In 17 of these, the CH-screening provided the first clue toward a diagnosis of CH-C. The age at inclusion (the day on which the TRH test was performed) ranged from 7–56 d (median 36 d), except for two patients, in whom investigations took place after the neonatal period; patient no. 16 was not referred and tested before the age of 2.1 yr, despite screening results indicative of CH-C; patient no. 11, born in India, was screened and tested at the age of 4 months, after the family had moved to The Netherlands. Patient no. 7 could not be completely tested in the neonatal period because T_4 supplementation had already been started at referral. TSH deficiency was confirmed by a TRH test at the age of 1 yr, after temporary discontinuation of T_4 supplementation. One patient (no. 1) with neonatal screening results and plasma TSH and free T_4 concentrations indicative of CH-C plus inadequately low plasma cortisol concentrations had multiple congenital defects associated with a chromosomal deletion del6pter→p22. He died of cardiorespiratory insufficiency before further endocrine tests could be performed.

One infant (patient no. 19), born to a mother with previ-

TABLE 2. Phase 2—Dynamic tests of thyrotropic function

Subject	TRH test					
	Age (d)	Free T_4 at t = 0 (ng/dl)	TSH at t = 0 (μU/ml)	TSH peak concentration (μU/ml) at time (min)	TSH at t = 180 (μU/ml)	Overall result
1						NA
2	25	0.58	3.0	49.0 (120)	32.0	A
3	17	0.58	10.0	72.0 (30)	58.0	A
4	49	0.61	6.6	30.4 (120)	18.1	A
5	39	0.64	2.6	10.4 (30)	2.6	A
6	29	0.65	1.5	16.8 (120)	14.0	A
7	358	0.52	1.7	10.6 (30)	2.0	A
8	49	0.65	4.0	42.4 (45)	18.4	A
9	15	0.66	4.2	30.2 (20)	18.8	A
10	19	0.71	2.3	7.8 (30)	3.9	A
11	137	0.78	<0.05	<0.05 (all)	<0.05	A
12	50	0.82	4.8	4.8 (0)	4.0	A
13	39	0.84	1.9	54.0 (120)	28.4	A
14	47	0.85	3.8	10.6 (30)	4.1	A
15	23	0.86	4.0	11.2 (15)	4.4	A
16	777	0.87	0.8	8.2 (60)	4.4	A
17	56	0.88	3.2	9.2 (30)	4.4	A
18	26	0.88	3.3	31.2 (45)	14.8	A
19	17	0.91	2.1	4.4 (30)	2.2	A
20	36	0.92	4.0	36.8 (60)	17.6	A
21	17	0.96	6.5	34.0 (30)	7.0	N
22	55	0.99	11.2	37.6 (30)	2.5	N
23	40	1.00	2.4	14.0 (30)	3.5	N
24	93	1.06	2.0	28.2 (30)	4.8	N
25	47	1.07	4.2	14.8 (20)	3.4	N
26	14	1.54	5.0	21.6 (30)	6.0	N

A, Abnormal; N, normal; NA, not assessed. Patient 1, no TRH test; baseline values: TSH, 5.9 μU/ml; T_4, 1.3 μg/dl (17 nmol/liter); free T_4, 0.33 ng/dl (4.3 pmol/liter); triiodothyronine, 20 ng/dl (0.3 nmol/liter) at age 7 d. Patient 9, No TSH determination at 180 min; 120 min value used instead. Conversion factors: TSH, 1 μU/ml = 1 mU/liter; T_4, 1 μg/dl = 12.87 nmol/liter; free T_4, 1 ng/dl = 12.87 pmol/liter; T_3, 1 ng/dl = 0.01536 nmol/liter.

TABLE 3. Phase 3—Assessment of the integrity of the hypothalamo-hypophyseal system; neuroradiologic imaging; clinical features

Patient	Adrenocorticotropic hormone axis					Somatotropic hormone axis					Gonadotropic hormone axis					MRI	Clinical features
	CRH test		ACTH test	Baseline		AT	Baseline				GnRH-test		Baseline				
	ACTH peak (pg/ml)	Cortisol peak (μg/dl)	Cortisol peak (μg/dl)	Random cortisol (μg/dl)	Overall result	GH peak (ng/ml)	IGF-I (ng/ml)	IGFBP-3 (μg/ml)	Retarded growth	Overall result	LH peak (mU/ml)	FSH peak (mU/ml)	Testosterone (ng/ml)	Estradiol (pg/ml)	Overall result		
1				10	NA					NA					NA	NA	1
2	120	4	4	1	A	6.2	27	0.54	+	A	<0.3	2.7		<5	A	0	
3			21	6*	A	8.5	30	0.30	+	A	5.4	20.0		11	N	NA	2
4	55	3		<2	A	1.6	23	0.69	+	A	<0.3	<0.5	<0.1		A	1	
5	90	38		17	N	15.9	49	0.79	−	N	12.0	14.0	0.4		N	2,3,4	3
6	78	6		1	A	4.0			+	A			0.2		A	1	
7	140	32		26	N	11.3	45	0.59	−	N	5.4	6.6	0.7		N	0	
8	120	16		8	N	30.8	27	1.36	+ (sec)	N†	6.4	22.0		13	N	1,2,4,5,6	4
9	24	2		2	A	<0.3	8		+	A	<0.3	1.3		<5	A	1,2	
10	75	18		4	B	20.7	48	0.99	−	N	10.0	4.4	2.2		N	5	5
11	165	25		33	N	<0.5	5	0.12	+	A					NA	0	6
12			13	<2	A	9.0	25	0.25	+	A	5.2	3.1	1.2		N	1,5	7
13	130	16	22	10	B	11.7	25	0.70	+ (sec)	N†	0.9	2.0	<0.1		A	1	
14	100	19		21	N	17.6	37	0.66	−	N	15.0	19.0	1.4		N	0	3
15	30	10	42	3	A	10.6	19	0.53	−	N	11.0	11.0	1.7		N	3	
16	260	48		24	N	2.7	15	0.52	+	A					NA	1	8
17	290	29		16	N	18.2	84	1.06	−	N	3.6	12.0	0.7		N	0	3
18	115	6		<2	A	4.6	13	0.61	+	A	0.6	1.5	<0.1		A	1	9
19	110	17		18	N				−	NA					NA	NA	
20	165	7	9	8	A	5.2	28	0.72	+	A	0.7	4.8		<5	A	1	

AT, Arginine test; estradiol, 17β-estradiol. Random cortisol: *, during hypoglycemia. Overall result: NA, Not assessed; N, normal; A, abnormal; B, borderline result; patients showed normal adrenocorticotropic function after 2-yr and 1-yr cortisol supplementation, respectively; (sec), patients 8 and 13 showed secondary growth retardation. MRI: 0, no manifest malformation; 1, posterior pituitary ectopia; 2, hydrocephalus internus; 3, hydrocephalus externus; 4, arachnoidal cyst(s); 5, corpus callosum agenesis; 6, bilateral periventricular nodular heterotopia. Clinical features: 1, del6pter->p22; 2, low-set ears, ventricular septal defect; 3, familial occurrence: patient no. 5, nephew and grandfather with same disorder, patients 14 and 17, younger brother with same disorder; 4, clubbed feet, father with cleft lip and palate; 5, brachycephaly; mother with hypothyroidism of unknown origin; 6, POU1F1 (Pit-1) deletion (49); 7, mental retardation, microcephaly, large ears, sparse hair, resembling Oliver-Mcfarlane syndrome; 8, multiple dysmorphias resembling fetal alcohol syndrome, small mandible, blepharophimosis; 9, (congenital) cataract, impaired hearing, clubbed feet, hypoplastic thumbs, atrial septal defect II. Conversion factors: ACTH, 1 pg/ml = 1 ng/liter; cortisol, 1 μg/dl = 27.59 nmol/liter; GH, 1 ng/ml = 2 mU/liter; IGF-I, 1 ng/ml = 1 μg/liter; IGFBP-3, 1 μg/ml = 1 mg/liter; LH, 1 mU/ml = 1 U/liter; FSH, 1 mU/ml = 1 U/liter; testosterone, 1 ng/ml = 3.467 nmol/liter; 17-β-estradiol, 1 pg/ml = 3.671 pmol/liter.

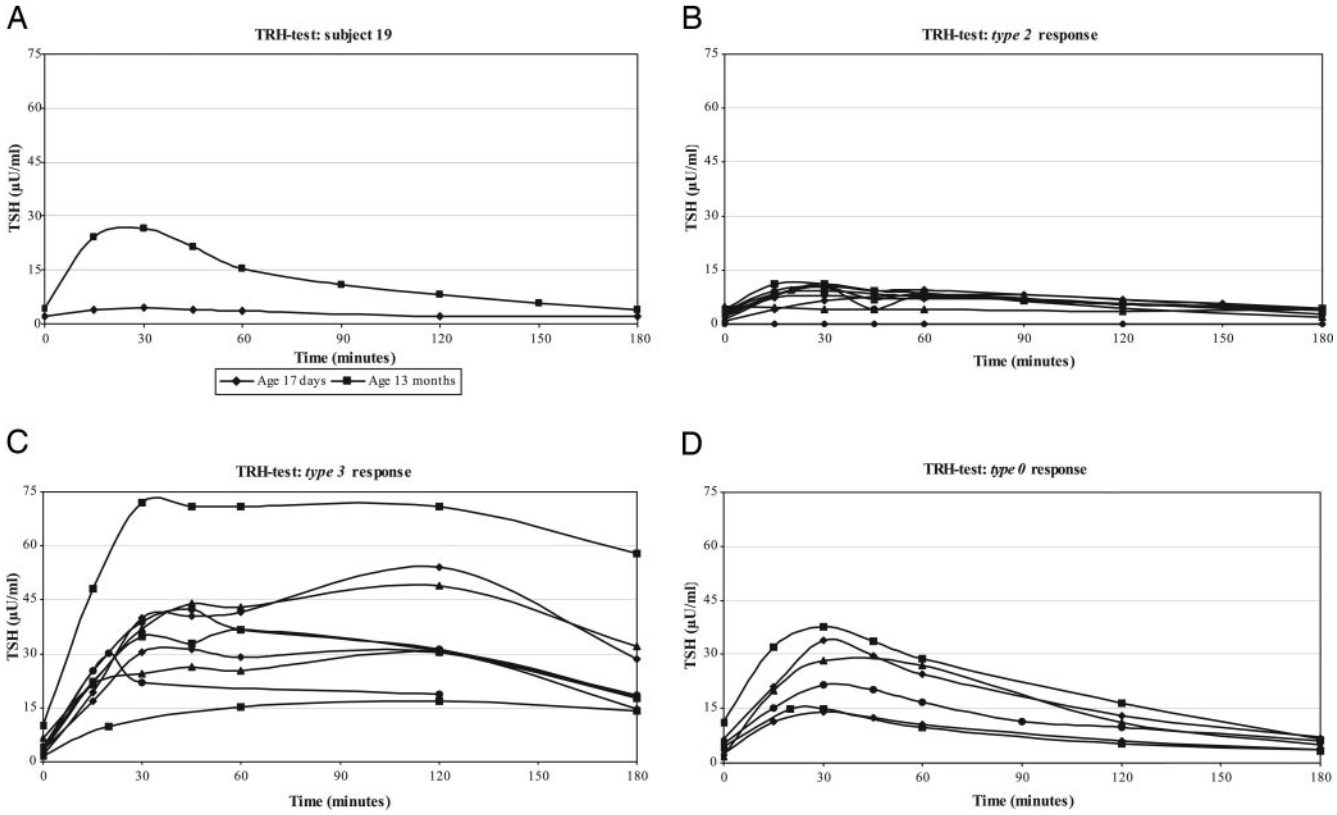

FIG. 2. Dynamic tests of thyrotropic function. A, Subject 19: type 2 response at the age of 17 d, type 0 response at the age of 13 months. B, Subjects 5, 7, 10–12, and 14–17: type 2 response. C, Subjects 2–4, 6, 8, 9, 13, 18, and 20: type 3 response. D, Subjects 21–26: type 0 response.

ously unnoticed Graves' disease, had abnormal TRH test results at the age of 17 d [TSH peak concentration of 4.4 μU/ml (4.4 mU/liter)]. By then, his free T_4 concentration had already increased to a near normal concentration of 0.91 ng/dl (11.7 pmol/liter) without medical intervention. Spontaneous restoration of thyroid function was awaited. From the age of 5 wk until the age of 13 months, TSH and free T_4 concentrations in plasma were within the normal range. A TRH test at the age of 13 months demonstrated normal TSH secretion (Fig. 2A) [TSH peak concentration, 26.4 μU/ml (26.4 mU/liter)]. Consequently, he was diagnosed with transient CH-C (29).

Six infants (patient nos. 21–26) with neonatal screening results indicative of CH-C appeared to have baseline plasma free T_4 concentrations exceeding 0.93 ng/dl (12 pmol/liter) at referral. Therefore, a diagnosis of CH-C was rejected, and we refrained from further testing after the initial tests of thyrotropic and adrenocorticotropic function. The putative causes for the false-positive screening results are summarized in Table 4. At follow-up, none of the surviving false-positive subjects have developed signs or symptoms of hypopituitarism. Among these subjects with false-positive screening results, the age of inclusion ranged from 14–93 d (median 44 d).

Neonatal CH screening results

Neonatal CH screening took place 5–15 d after birth (median 6 d), except for patient no. 11, who was screened at the age of 130 d, after he had moved from India to The Netherlands. The patients' TSH concentrations in dried heel puncture blood spots ranged from below detection level to 7 μU/ml plasma, T_4 concentrations ranged from 0.6–11.3 μg/dl (8–145 nmol/liter), and TBG concentrations ranged

TABLE 4. Putative explanations for false-positive screening results (subjects 21–26)

Subject	Putative explanation for false-positive screening results
21	Parenchymal hemorrhage in glandula pinealis region and intraventricular hemorrhage, Ohtahara epilepsia; transient central hypothyroidism, possibly due to elevated intracranial pressure or arterial insufficiency. Alternatively, the abnormal screening results could be due to a "sick euthyroid state."
22	Transient mild central hypothyroidism of unknown origin; normal TSH and free T_4 concentrations at long-term follow-up.
23	Low normal (free) T_4 values, but adequate TSH response to TRH; no clinical signs of hypothyroidism, normal growth and development at follow-up: normal variant?
24	Transient mild central hypothyroidism of unknown origin; normal TSH and free T_4 concentrations at long-term follow-up.
25	Perinatal streptococcal meningoencephalitis; sinus thrombosis, extensive infarction a. cerebri posterior area, slightly dilated ventricles; transient central hypothyroidism, possibly due to elevated intracranial pressure or arterial insufficiency.
26	TBG deficiency and erroneous initial free T_4 determination.

from 9.9–31.8 μg/dl (183–589 nmol/liter). All patients except nos. 8 and 19 had T_4 to TBG ratios below the cut-off level of 8.5, previously indicated for discrimination of CH-C patients from false-positives in the screening population (15). Patients 8 and 19 were referred on the basis of low screening T_4-SDS and consecutive plasma free T_4 concentrations less than 0.93 ng/dl (12 pmol/liter) and plasma TSH concentrations less than 15 μU/ml (15 mU/liter; Table 1).

Investigation of the thyrotropic hormone axis

All infants with baseline plasma free T_4 concentrations less than 0.93 ng/dl (12 pmol/liter) exhibited type 2 (n = 9) or type 3 (n = 9) responses to TRH (Fig. 2, B and C). The cut-off level for diminished (type 2) TSH response could be estimated at 14 μU/ml (14 mU/liter; Table 2). The patients' type 2 and type 3 responses, expressed as area under the curve, were significantly different ($P = 0.001$ for type 2; $P = 0.013$ for type 3 by the Mann-Whitney *U* test) from the responses of subjects 21–26, who exhibited type 0 responses (Fig. 2D).

Investigation of the ACTH axis

Nine of 19 patients tested (47%) were found to be deficient, whereas eight had clearly adequate results and two (patients 10 and 13) had borderline results and were given cortisol-supplementation for 1 and 2 yr, respectively. After discontinuation of supplementation, they had normal adrenocorticotropic function, as evidenced by a normal circadian cortisol rhythm.

Of the nine patients found deficient, four showed impaired response of both ACTH and cortisol to CRH. Another three patients (nos. 2, 18, and 20) showed adequate ACTH response, but impaired cortisol response. ACTH tests in two of the latter infants resulted in impaired cortisol response accordingly. Two patients were considered too small (no. 12) or too ill (no. 3) to undergo a CRH test, for which a relatively large amount of blood is required. Instead, ACTH tests were performed. The results of random plasma cortisol samples taken in all subjects were in accordance with the function test results (Table 3).

Investigation of the somatotropic hormone axis

After 5 yr of follow-up, 12 of 18 (67%) patients with permanent CH-C alive [from the initial 20 patients, one (no. 1) had died, and one (no. 19) had transient CH-C] have shown retarded growth. Arginine tests, performed at the age of 3 months when euthyroid status had been accomplished by T_4 supplementation, were abnormal in 10 of those infants and normal in all infants with normal growth (Table 3). Two infants (nos. 8 and 13) with normal arginine test results showed growth retardation around the age of 2.5 yr. Both had repeated GH stimulation tests that yielded severely impaired GH release after stimulation with arginine, GHRH (no. 8) and clonidine (no. 13), respectively. Baseline growth factors were measured at the age of 3 months. Significant differences between children with normal *vs.* retarded growth (exclusive of subjects 8 and 13) were observed in arginine test results ($P = 0.001$ by the Mann-Whitney *U* test) as well as baseline plasma concentrations of IGF-I and IGFBP-3 at age 3 months ($P = 0.001$ and $P = 0.011$, respectively).

Investigation of the gonadotropic hormone axis

Six of 15 patients tested (40%) did not reach plasma peak concentrations of LH exceeding 3 mU/ml (3 U/liter) and FSH exceeding 6 mU/ml (6 U/liter; girls) or 3 mU/ml (3 U/liter; boys) after infusion of GnRH. Two of those had hypogenitalism. All six hypogonadotropic patients had baseline morning plasma concentrations of testosterone (boys) less than 0.6 ng/ml (2 nmol/liter) or 17-β-estradiol (girls) less than 11 pg/ml (40 pmol/liter) at age 3 months. One patient (no. 6) who was not subjected to a GnRH test did have an abnormally low baseline plasma testosterone concentration (Table 3).

Investigation of posterior pituitary function

None of the subjects studied had symptoms indicating disturbance of posterior pituitary function.

MRI of the brain

Nine of 17 patients (53%) who underwent MRI had a small anterior pituitary lobe, located in the sella turcica, invisible or very thin pituitary stalk, and ectopic posterior pituitary lobe, located at the median eminence in the floor of the third ventricle (Fig. 3). Six patients had cerebral malformations, such as agenesis of the corpus callosum and bilateral periventricular nodular heterotopia and/or hydrocephalus. All patients with posterior pituitary ectopia had multiple pituitary hormone deficiency (Table 3).

Discussion

In the first 2 yr after introduction of the novel neonatal CH screening protocol in The Netherlands, we identified 19 cases of permanent CH-C among 385,000 infants screened, representing 13.5% of the total number of patients with permanent CH (n = 141) (30–32) as detected by neonatal screening.

Initial estimates of the prevalence of CH-C among live born children in the United States and Canada ranged from 1:110,000 to 1:29,000 (33–35). In a similar retrospective study of the 1981–1989 screening population in The Netherlands, we estimated a prevalence of 1:26,000 (36). Three fourths of these children turned out to have multiple pituitary hormone deficiency. Delayed detection and incomplete diagnosis of the hormonal deficiencies in question resulted in significant morbidity such as severe hypoglycemia and neonatal hepatitis and a mortality rate as high as 14% (36). Because all deficient hormones can readily be supplemented, timely diagnosis significantly improves the outcome (37). The prevalence of 1:20,263 live born neonates (95% confidence interval, 1:12,976 to 1:33,654), as estimated in the present study, is higher than previously reported (33–36), presumably due to the fact that this is the first representative and unbiased cohort, efficiently detected. In a recent evaluation of the 1995–2000 period, we estimated a prevalence of 1:16,404 (95% confidence interval, 1:13,174 to 1:21,173) at an estimated detection rate of 91.6% (38). Because it is unlikely that the prevalence of CH-C in The Netherlands is significantly dif-

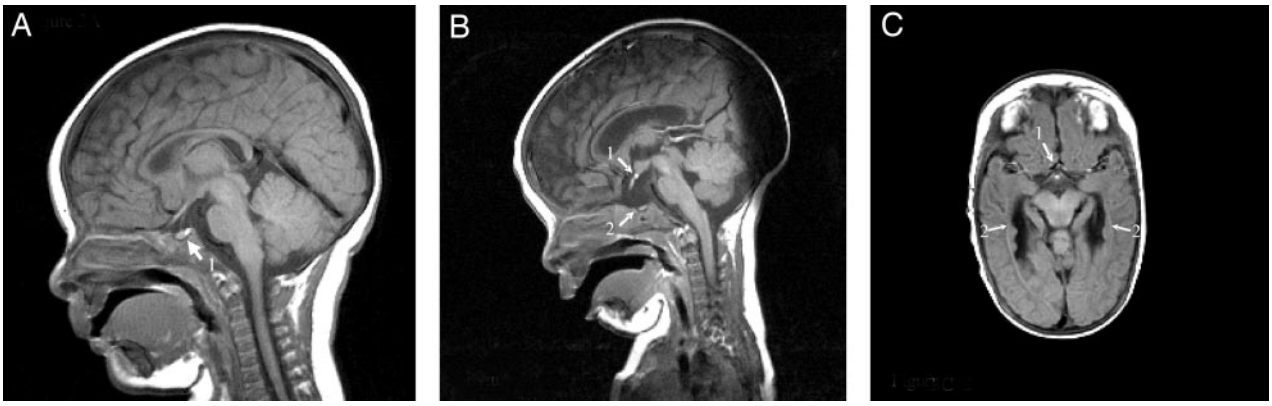

FIG. 3. MRI of the brain. A, T1-weighted spin echo midsagittal image of a 3-month-old boy, screened for neonatal hemangiomatosis. Normal configuration of pituitary and pituitary stalk. *Arrow* points at sella turcica with pituitary anterior lobe (*gray*) and posterior lobe (*bright white*). B and C, T1-weighted spin echo images of patient no. 8 at age 3 months. B, Midsagittal image showing ectopic posterior pituitary lobe (*arrow 1*) at the median eminence in the floor of the third ventricle and small anterior pituitary lobe in undersized sella turcica (*arrow 2*). No manifest pituitary stalk. C, Transversal image showing posterior pituitary ectopia (*arrow 1*) and bilateral periventricular nodular heterotopia (*arrows 2*).

ferent from that in North America, we presume that in prior studies (33–36) both the mildest and the most severe cases have been missed, similar to the situation in 21α-hydroxylase deficiency in the prescreening era (39, 40).

An anticipated Achilles' heel of our study is the fact that normal values for endocrine function tests for this specific age group are largely unavailable. However, it turned out that patients with CH-C could be discriminated very well from the infants with false-positive screening results on the basis of their TRH test results. Further evidence was acquired from the fact that the majority of CH-C patients (78%) had multiple pituitary hormone deficiency and 53% had a pituitary malformation as visualized by MRI. All patients had baseline plasma free T_4 concentrations less than 0.93 ng/dl (12 pmol/liter) at referral.

TRH tests of CH-C patients show either diminished (type 2) (19–21), or slightly delayed but excessive increase and delayed decrease of the TSH plasma concentration (type 3) (19, 20). Generally, type 2 responses are considered to reflect disturbance at the pituitary level, whereas type 3 responses reflect disturbance at the hypothalamic level. Accordingly, the clinical pictures associated with these responses are termed secondary/pituitary and tertiary/hypothalamic hypothyroidism. Remarkably, seven of the nine patients with the pituitary malformation posterior pituitary ectopia (Table 3) exhibit type 3 responses. Apparently, not the disturbance of the pituitary itself, but instead the disturbance of the interrelation between hypothalamus and (anterior) pituitary gland causes the pituitary deficiency.

Consequently, our study confirms other reports (41–43) that the distinction of secondary and tertiary hypothyroidism is improper. Therefore, we recommend the use of the term "congenital hypothyroidism of central origin" (CH-C) for neonates with apparent TSH deficiency (44) and congenital disorders of the hypothalamo-hypophyseal system to cover multiple pituitary hormone deficiency.

Forty-seven percent of the CH-C patients had evidence of glucocorticoid deficiency. Remarkable was our observation that three patients exhibited substantial rise of plasma ACTH but not cortisol concentrations in response to CRH. This phenomenon, indicative of adrenocortical hypoplasia as a result of long-time insufficient stimulation by ACTH, has not been described before.

Sixty-seven percent of the CH-C patients had evidence of GH deficiency, as shown by growth retardation and catch-up growth after installment of GH treatment. Two of these patients had normal test results at the age of 3 months. They showed retarded growth in the third year of life and at repeated testing proved severely GH deficient.

Gonadal function cannot be definitively assessed at this age. However, we speculated that the temporary activity of the gonadotropic axis in infancy (17, 18) would enable function testing at the age of 3 months. It will take another decade to validate the results, but GnRH test results and baseline testosterone and 17β-estradiol concentrations clearly divided the study population into two groups. Nine of 15 infants tested had results comparable to those in Tanner stages 2–4. Six infants (40%) had baseline (morning) plasma concentrations of testosterone less than 0.6 ng/ml (2 nmol/liter) or 17-β-estradiol less than 11 pg/ml (40 pmol/liter) and did not reach LH concentrations above 3 mU/ml (3 U/liter) and FSH concentrations above 6 mU/ml (6 U/liter; girls) or 3 mU/ml (3 U/liter; boys) after infusion of GnRH.

None of the patients had symptoms indicating disturbance of posterior pituitary function neonatally or at follow-up. However, partial diabetes insipidus cannot be ruled out, because it has been shown that, even in the absence of polyuria, polydipsia, or nocturnal enuresis, the vasopressin response to osmolar stimuli might very well be subnormal in patients with (congenital) hypopituitarism and posterior pituitary ectopia, suggesting damage in the hypothalamic vasopressin secreting centers (45).

The MRI studies performed in 17 of the patients revealed nine cases (53%) of posterior pituitary ectopia, a pituitary malformation often associated with multiple pituitary hormone deficiency (5, 46, 47). All of these infants had deficiencies of one or more pituitary hormones besides TSH. In fact, the emergence of a second deficiency of anterior pituitary function was predicted by the finding of posterior pituitary ectopia in patient no. 8, who initially had TSH deficiency only.

In conclusion, our data indicate that infants with permanent CH-C, making up 13.5% of all permanent CH patients, can be detected very well in a T_4-based neonatal CH screening program, extended with TSH and TBG determinations. The majority of patients detected have multiple pituitary hormone deficiency, a life-threatening disorder. Therefore, neonatal screening should be followed by assessment of the integrity of the hypothalamo-hypophyseal system without delay. In this respect, the protocol here presented proved feasible and effective. CH-C warrants early detection and timely treatment, and thus putative reduction of mortality and severe morbidity, such as neuroglycopenia and consequent irreversible damage to the central nervous system. The estimated prevalence and the severity of pituitary dysfunction of this treatable disorder call for explicit attention for this entity of CH in neonatal screening programs worldwide.

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