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Hypogonadotropic Hypogonadism in Infants with Congenital Hypopituitarism: A Challenge to Diagnose at an Early Stage

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Key Words

Congenital hypogonadotropic hypogonadism · Congenital hypopituitarism · Gonadotropic surge · Anti-Müllerian hormone · Inhibin B

Abstract

Background: Combined pituitary hormone deficiency (CPHD) presents a wide spectrum of pituitary gland disorders. The postnatal gonadotropic surge provides a useful period to explore the gonadotropic axis for assessing the presence of congenital hypogonadotropic hypogonadism (CHH). ***Aim:*** To explore the functioning of the hypothalamic-pituitary-gonadal axis in the postnatal gonadotropic surge for an early diagnosis of CHH in newborns or infants suspected of having CPHD. ***Subjects and Methods:*** A cohort of 27 boys under 6 months and 19 girls under 24 months of age with suspected hypopituitarism was studied. Serum concentrations of LH, FSH, testosterone, inhibin B, anti-Müllerian hormone (AMH) and estradiol were measured, and male external genitalia were characterized as normal or abnormal (micropenis, microorchidism and/or cryptorchidism). ***Results:*** CPHD was confirmed in 36 out of 46 patients. Low LH and testosterone levels were found in 66% of the hypopituitary males, in significant association with the presence of abnormal external genitalia. This abnormality had a positive predictive value of 93% for CHH. No significant association was observed between serum FSH, AMH and inhibin B and the patient's external genitalia. ***Conclusion:*** In newborn or infant boys with CPHD, LH and testosterone concentrations measured throughout the postnatal gonadotropic surge, together with a detailed evaluation of the external genital phenotype, facilitate the diagnosis of CHH at an early stage.

Introduction

Normal fetal development of the gonads involves differentiation from the urogenital ridge through a complex genetic and hormonal pathway [1]. In boys, during the first trimester, placental human chorionic gonadotropin, acting through LH/human chorionic gonadotropin receptors in the Leydig cells, is the main regulatory factor for the production of fetal testosterone and insulin-like factor 3. These are involved in the transabdominal phase of testicular descent [1]. At the end of the first trimester, when the fetal hypothalamic-pituitary axis function is

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well established, gonadotropins take over testicular regulation, leading to testicular and penile enlargement and full testicular descent [2]. Consequently, a normal hypothalamic-pituitary-gonadal axis function is essential for complete development of external male genitalia at birth [3]. Conversely, in girls, this process is independent of hormone secretion; hence, congenital hypogonadotropic hypogonadism (CHH) is not expected to affect the normal development of female external genitalia [4].

In boys, gonadotropins play an important role in early postnatal Leydig cell proliferation and germ cell differentiation. LH stimulates Leydig cell testosterone production, whereas FSH stimulates Sertoli cell proliferation and secretion of anti-Müllerian hormone (AMH) and inhibin B (Inh-B) [5]. During the first week of life, concentrations of LH, FSH and testosterone are temporarily low; the AMH concentration is measurable, while the Inh-B concentration reaches levels as high as those observed in pubertal boys [6]. The AMH concentration remains high until puberty. The high Inh-B concentration in circulation remains unchanged up to 6 months of age, progressively dropping to reach the nadir at approximately 4 years of age and to increase again at puberty [7–9].

Within the first months of life, the testicular volume increases due to mitotic activity of both germ and Sertoli cells together with an increment in seminiferous tubule length [10], making this period potentially critical for future spermatogenic function and fertility [11, 12]. The pattern of circulating gonadotropins shows a marked sexual dimorphism in LH and FSH, with a clear preponderance of LH in boys and FSH in girls from fetal life up to puberty [4, 6].

By the age of 6 months in males and 2–3 years in females, the development of the intrinsic central inhibitory tone exerted on the gonadotropin-releasing hormone (GnRH) pulse generator progressively decreases gonadotropin secretion, reaching low prepubertal concentrations that persist until the onset of puberty [3].

Nowadays, the incidence of congenital hypopituitarism is known to affect 1:4,000 to 1:10,000 newborns, and the development of combined pituitary hormone deficiency (CPHD) is attributed to defects in transcription factors and signaling pathways involved in pituitary gland organogenesis [13]. The prevalence of CHH in CPHD remains unknown.

For this reason, the postnatal gonadotropin surge may be an extraordinarily useful period of time to explore the integrity of the pituitary-gonadal axis to either confirm or exclude the presence of CHH in CPHD patients, offering the advantage of a prepubertal diagnosis.

The aim of this study was to explore the hypothalamic-pituitary-gonadal axis function throughout the physiological postnatal gonadotropic surge for an early diagnosis of CHH in newborns or infants with suspected congenital hypopituitarism.

Patients and Methods

Patients

The hypothalamic-pituitary axis function was studied in boys aged under 6 months and girls under 24 months, who had been referred to our unit from June 2005 to July 2014 to rule out congenital hypopituitarism.

After clinical examination, external genitalia in boys were classified as normal or abnormal, the latter defined by the existence of micropenis [length of penis less than –2.5 standard deviation score (SDS)] [14, 15], microorchidism (testicular volume <1 ml measured with the Prader orchidometer) and/or cryptorchidism.

CPHD was defined by the presence of two or more of the following pituitary hormone deficiencies: thyroid-stimulating hormone (TSH) deficiency: free thyroxine <1.0 ng/dl with low or normal TSH levels (TSH ≤10 mU/l in patients aged under 2 months and ≤6.5 mU/l in older infants); ACTH deficiency: low basal serum cortisol (<1.1 μg/dl in patients under 2 months of age, <2.1 μg/dl in patients between 2 and 6 months, and <6 μg/dl in older infants) [16] or serum cortisol <20 μg/dl under hypoglycemia, associated with low or normal plasma ACTH; GH deficiency: basal GH <2.0 ng/ml during the first days of life [17] or <10 ng/ml under hypoglycemia. Older infants were diagnosed with GH deficiency on the basis of abnormal growth velocity and maximal GH-stimulated levels of <6.0 ng/ml after two pharmacological stimulation tests. Prolactin deficiency was considered with serum levels <2.5th centile for sex and age. Central diabetes insipidus was diagnosed when polyuria was associated with a urinary:plasma osmolarity ratio of <1.5 and the patient had a plasma osmolality of >300 mosm/l.

CHH was assumed in boys aged between 15 days and 6 months when serum LH and testosterone were <5th centile (<0.8 IU/l and <30 ng/dl, respectively) [8]. In girls from the age of 15 days to 2 years, CHH was assumed when FSH levels were <1.0 IU/l [6, 9, 18, 19].

Patients were grouped according to their pituitary function (normal or congenital hypopituitarism). The latter was further classified according to the presence or absence of hypogonadism (fig. 1).

Methods

Hormone Assays

Serum TSH, FT4, cortisol, prolactin, gonadotropins, testosterone and estradiol concentrations were measured with an electrochemiluminescence assay (Roche Diagnostics GmbH, Mannheim, Germany) using a Cobas e411 analyzer according to the manufacturer's instructions. Serum GH, IGF-I and plasma ACTH were measured with a two-site chemiluminescence immunometric assay (IMMULITE® 2000 system; Siemens Healthcare Diagnostics Products Ltd., Gwynedd, UK). Intra- and interassay coefficients of variation (CV) were <4% for GH, <5.5% for IGF-I, <3.6% for prolactin, <2.9% for TSH, <1.9% for FT4, <4.0% for cortisol and <7.0% for ACTH.

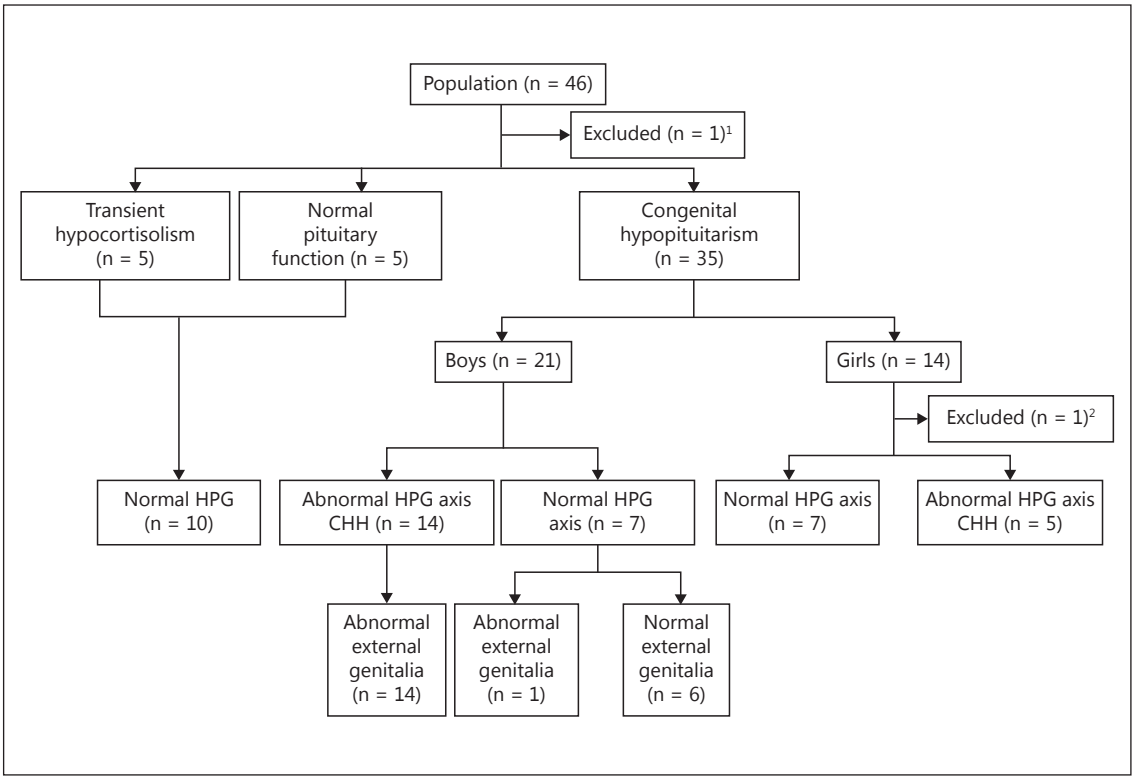

Fig. 1. Evaluation of pituitary function in patients referred to exclude congenital hypopituitarism. HPG axis = Hypothalamic-pituitary-gonadal axis. [1] Lost to follow-up. [2] Gonadotropins were not available.

The sensitivity of the assay for both LH and FSH was 0.10 IU/l. The WHO second IS 80/552 and the WHO second IRP 78/549 were used as LH and FSH standards, respectively. Intra- and interassay CV were 1.1 and 1.8% for LH and 1.0 and 4.2% for FSH, respectively. Testosterone assay sensitivity was 10 ng/dl, and intra- and interassay CV were 2.4 and 2.6%, respectively. Estradiol assay sensitivity was 10 pg/ml, and intra- and interassay CV were <3.8 and < 6.5%, respectively. When serum gonadotropins, testosterone or estradiol concentrations were undetectable, the value of the limit of detection was attributed. Serum AMH was measured using the AMH/Müllerian-inhibiting substance ELISA kit (Immunotech-Beckman, Marseilles, France). The limit of detection, i.e. the lowest AMH concentration significantly different from the calibrator zero, was 2.53 pmol/l; intra- and interassay CV were 5.3 and 8.7%, respectively, for a serum AMH concentration of 35 pmol/l, and 4.9 and 7.8% for a serum AMH concentration of 1,100 pmol/l. Serum Inh-B was measured by ELISA (Inhibin B Gen II ELISA, Beckman-Coulter, Inc., Webster, Tex., USA). Assay sensitivity was 10 pg/ml, and the intra- and interassay CV were 4.0 and 5.6%, respectively.

Brain Magnetic Resonance Imaging

Patients with CPHD underwent brain magnetic resonance imaging (MRI) for the evaluation of cerebral and hypothalamic pituitary abnormalities.

Statistics

Data are expressed as the mean ± SD. Repeated-measures one-way analysis of variance (ANOVA) with the Tukey-Kramer test as posterior analysis was used to evaluate differences between groups. Fisher's exact test was used to assess the association of low gonadotropins and testosterone, with the presence of abnormal external genitalia. The level of significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, Calif., USA. www.graphpad.com).

The study was performed according to the Helsinki Declaration and approved by the local Institutional Review Board. All parents of the participants provided informed consent.

Results

Forty-six patients (27 boys and 19 girls) were admitted to rule out congenital hypopituitarism. Referral of patients was mainly based on the presence of neonatal hypoglycemia and/or neonatal cholestasis. Genital abnormalities were the cause of referral in 7 out of 27 boys, 5 of whom also presented hypoglycemia and/or cholestasis.

Table 1. Pituitary function, external genitalia phenotype and gonadal axis evaluation in boys

Patient No.	GA, weeks	Cause of referral	Pituitary function	Compromised axis	External genitalia phenotype	Hormonal assessment					
						age, months	LH, IU/l	FSH, IU/l	Testosterone, ng/dl	AMH, pmol/l	Inh-B, pg/ml
1	40	hypoglycemia	hypopituitarism	ACTH/TSH/GH/high PRL	micropenis, b-microorchidism	1.0	<0.10	<0.10	<10	740	149.4
2	39	hypoglycemia, microorchidism	hypopituitarism	ACTH/TSH/GH	micropenis, b-microorchidism	1.0	<0.10	0.20	<10	554	77.3
3	35	hypoglycemia, cholestasis	hypopituitarism	ACTH/TSH/GH/high PRL	micropenis, b-microorchidism	3.0	<0.10	<0.10	<10	200	31.7
4	40	hypoglycemia, cholestasis	hypopituitarism	ACTH/TSH/GH	micropenis, b-microorchidism	1.5	0.70	2.2	<10	443	83.3
5	38	hypoglycemia, cholestasis	hypopituitarism	ACTH/TSH/GH	b-cryptorchidism	2.0	0.10	0.10	<10	750	55.6
6	38	hypoglycemia	hypopituitarism	ACTH/TSH/low IGF-I	micropenis, b-cryptorchidism	6.0	0.20	4.1	<10	601	124.1
7	40	hypoglycemia, micropenis, microorchidism	hypopituitarism	ACTH/TSH/GH	micropenis, b-cryptorchidism.	5.0	<0.10	0.30	<10	153	20.2
8	38	micropenis, microorchidism	hypopituitarism	ACTH/TSH/high PRL/low IGF-I	micropenis, b-cryptorchidism	3.0	0.10	1.4	<10	603	89.6
9	40	hypoglycemia, micropenis	hypopituitarism	ACTH/TSH/GH	micropenis	1.0	0.10	0.30	<10	94	NA
10	33	cholestasis	hypopituitarism	ACTH/TSH/GH	micropenis, b-microorchidism	6.0	0.70	1.8	<10	557	NA
11	37	micropenis	hypopituitarism	GH/TSH	micropenis, u-cryptorchidism, b-microorchidism	6.0	<0.10	<0.10	<10	NA	NA
12	40	micropenis	hypopituitarism	ACTH/TSH/low IGF-I	micropenis, b-microorchidism	1.0	0.10	0.40	13	744	76.1
13	40	hypoglycemia, micropenis	hypopituitarism	ACTH/TSH/ADH/low IGF-I	micropenis, u-cryptorchidism	0.5	0.20	1.3	<10	332	42.1
14	34	hypoglycemia	hypopituitarism	ACTH/TSH/low IGF-I/high PRL	micropenis	0.4	<0.10	0.20	<10	222	16.2
15	37	hypoglycemia, cholestasis	hypopituitarism	ACTH/TSH/high PRL	b-microorchidism	1.0	4.1	2.1	142	NA	214.4
16	38	hypoglycemia	hypopituitarism	ACTH/TSH/GH/PRL	normal	1.5	9.5	7.9	440	995	276.9
17	37	hypoglycemia	hypopituitarism	ACTH/GH	normal	1.5	2.8	1.7	122	800	NA
18	37	hypoglycemia, hepatitis	hypopituitarism	ACTH	normal	0.5	10.5	5.1	132	859	84.4[a]
19	36	hypoglycemia, cholestasis	hypopituitarism	ACTH/TSH/low IGF-I	normal	0.9	5.0	1.6	155	716	298.4
20	39	hypernatremia	hypopituitarism	ACTH/TSH/ADH/low IGF-I	normal	0.75	6.02	1.32	55	796	NA
21	39	hypoglycemia, cholestasis	hypopituitarism	ACTH/TSH/low IGF-I	normal	2.1	7.48	3.62	90	533	NA
22	40	hypoglycemia, cholestasis	normal		normal	1.9	4.4	2.9	155	NA	NA
23	34	hypoglycemia, cholestasis	normal		normal	1.0	3.5	2.7	383	NA	NA
24	37	hypoglycemia	t-hypocortisolism		normal	1.5	9.2	2.0	255	505	NA
25	36	hypoglycemia, cholestasis	t-hypocortisolism		normal	1.8	4.1	1.9	190	388	NA
26	26	hypoglycemia, cholestasis	t-hypocortisolism		normal	6.0	4.3	2.2	111	NA	NA
27	40	hypoglycemia, cholestasis	t-hypocortisolism		normal	1.8	4.2	2.7	113	577	NA
					normal reference value		>0.8	>0.45	>30	1–3 months >424 ≥3–6 months >749	>80.0

GA = Gestational age; PRL = prolactin; b = bilateral; u = unilateral; t-hypocortisolism = transient hypocortisolism; NA = not available.
[a] Performed at 3 years (normal range 85.9–219.5 pg/ml).

The mean gestational age was 37.0 ± 3.4 weeks (range 26–40), and the mean birth weight was 2,878 ± 891 g (range 1,030–4,120). The age at referral was 1.8 ± 1.7 months (range 0.4–6.0) for boys and 5.1 ± 3.9 months (range 0.6–23.0) for girls.

Based on clinical and endocrinological evaluation, 34 patients (73.9%) were diagnosed with congenital hypopituitarism, 5 (10.8 %) with isolated transient hypocortisolism and the remaining 5 (10.8 %) with normal pituitary function. Among the patients with congenital hypopituitarism, 32 (94%) had CPHD; 1 boy had isolated ACTH deficiency, and 1 girl had isolated GH deficiency.

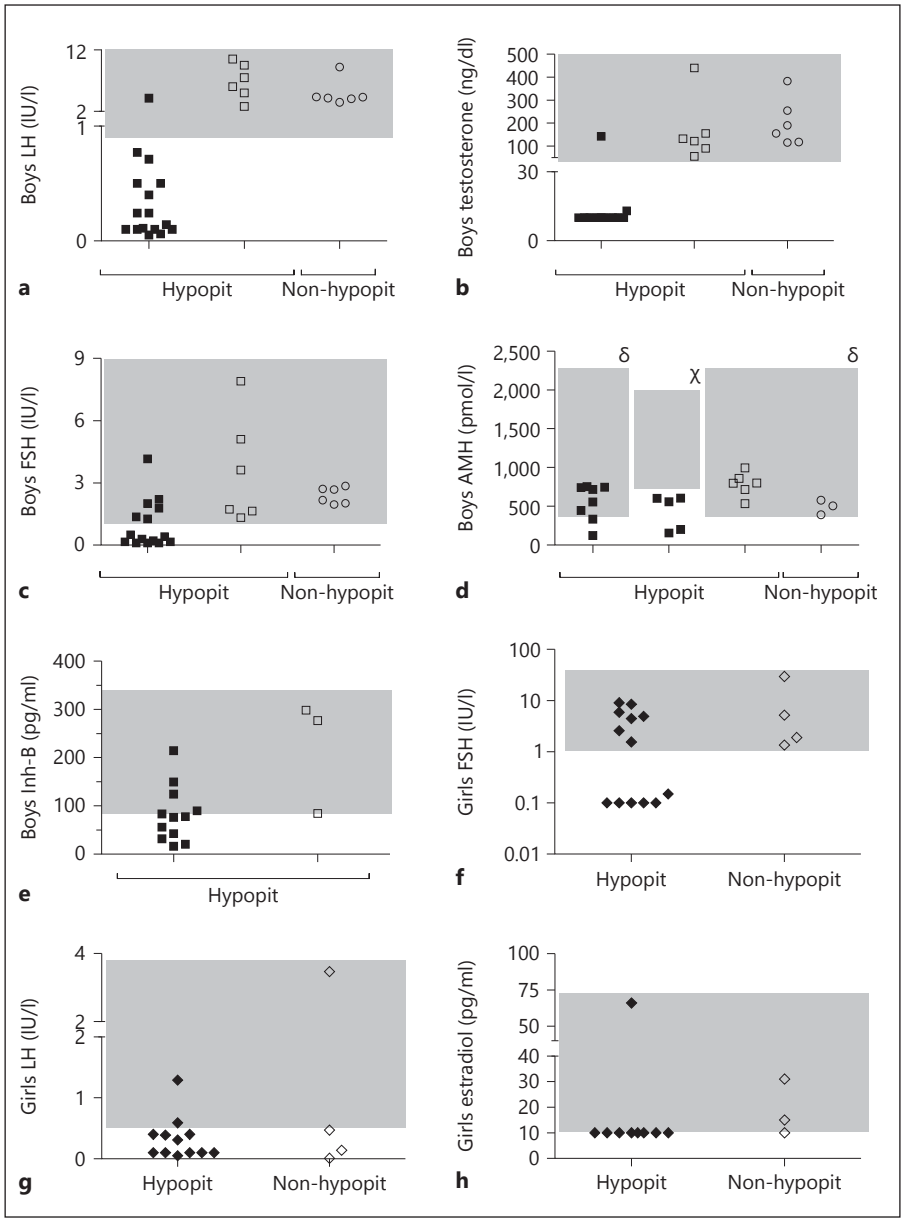

Fig. 2. Hormonal determinations in boys (**a–e**) and girls (**f–h**). δ = Normal range for <3 months of age; χ = normal range for ≥3 months of age. Shaded fields express the normal range for the age. Boys: hypopituitary (hypopit) patients with abnormal genitalia (■), normal genitalia (□) and non-hypopituitary (non-hypopit) patients (○). Girls: hypopituitary patients (◆) and non-hypopituitary patients (◇).

Congenital Hypopituitarism

Boys

Congenital hypopituitarism was confirmed in 21 out of 27 boys (77.8%). Upon admission, an evaluation of the external genitalia in patients with hypopituitarism revealed abnormal external genitalia in 15 out of 21 boys. Twelve had more than one genital anomaly (table 1).

Assessment of the gonadal function revealed low concentrations of both LH and testosterone in 14 out of 21 patients, thus suggesting the diagnosis of CHH. Concentrations of FSH, AMH and Inh-B were below the normal range in 8 out of 14 (57%), 7 out of 13 (54%) and 7 out of 12 (58%) patients, respectively. Normal serum concentrations of LH, FSH, testosterone, AMH and Inh-B were found in all boys with normal external genitalia (patients No. 16–21) and in 1 boy with genital anomalies (fig. 1; table 1).

In hypopituitary patients, a significant association was observed between abnormal genitalia and low abnormal concentrations of LH and testosterone (Fisher's exact test, $p < 0.0001$). The presence of abnormal external gen-

Table 2. Pituitary function and gonadal axis evaluation in girls

Patient No.	GA, weeks	Admission		Pituitary function	Compromised axis	Hormonal assessment			
		cause of referral	age, months			age, months	LH, IU/l	FSH, IU/l	estradiol, pg/ml
1	34	cholestasis	1	hypopituitarism	GH/ACTH/TSH/PRL	1	0.1	0.1	NA
2	36	hypoglycemia, cholestasis	1	hypopituitarism	GH/ACTH/TSH	1	0.05	0.1	NA
3	40	hypoglycemia	0.6	hypopituitarism	ACTH/GH	0.6	0.4	0.1	NA
4	NA	microcephaly, familial hypopituitarism	5	hypopituitarism	ADH/TSH/GH?	5	0.4	1.55	<10
5	39	hypoglycemia, short stature	11	hypopituitarism	GH	20	0.1	4.93	<10
6	37	hypoglycemia	2	hypopituitarism	GH/ACTH/TSH/PRL	24	0.1	0.1	<10
7	39	diabetes insipidus	4	hypopituitarism	ADH/GH	24	0.59	9.1	<10
8	38	midline defect	23	hypopituitarism	ACTH/high PRL/low IGF-I	23	0.1	4.46	<10
9	38	macrosomy, hypernatremia	6	hypopituitarism	ADH/low IGF-I	6	1.29	8.46	<10
10	40	septo-optic dysplasia	4	hypopituitarism	ADH/low IGF-I	0.8	0.31	5.91	<10
11	39	hypoglycemia	0.6	hypopituitarism	ACTH, GH	1	0.1	0.1	NA
12	39	hypoglycemia, hypernatremia	0.6	hypopituitarism	ACTH/TSH/ADH	9	0.39	2.59	<10
13	37	septo-optic dysplasia	1.0	hypopituitarism	ACTH/TSH/ADH/low IGF-I	1.0	0.1	0.25	66
14	30	cholestasis	4.4	normal		4.4	0.47	5.2	10
15	33	hypoglycemia, cholestasis	8.75	normal		8.75	0.14	1.9	31
16	28	cholestasis	3.75	normal		3.75	3.47	29.61	NA
17	40	hypoglycemia, cholestasis	2.6	t-hypocortisolism		2.6	0.01	1.35	15

GA = Gestational age; PRL = prolactin; t-hypocortisolism = transient hypocortisolism; NA = not available.

italia had a positive predictive value of 93% [95% confidence interval (CI) 0.68–0.99], 100% sensitivity (95% CI 0.76–1.00) and 85% specificity (95% CI 0.42–0.99) for CHH, whereas the existence of normal external genitalia excluded CHH in all cases (negative predictive value 100%, 95% CI 0.73–1.00). No significant association was observed between external genital phenotype and serum concentrations of FSH ($p > 0.05$), AMH ($p > 0.05$) and Inh-B ($p > 0.05$).

Patients with CHH (low LH and testosterone) had significantly lower serum AMH and Inh-B concentrations than patients without CHH (461 ± 236 vs. 817 ± 116 pmol/l, $p < 0.01$, and 69.6 ± 42 vs. 263.0 ± 46.3 pg/ml, $p = 0.01$, respectively). Nevertheless, neither AMH nor Inh-B had a diagnostic significance for CHH. A low concentration of FSH was only found in 9 patients with CHH.

Boys with hypopituitarism and normal external genitalia (n = 6) had a normal pituitary-gonadal axis function (fig. 2a–e): serum LH and testosterone concentrations were similar to those observed in patients without hypopituitarism (n = 6; LH 6.88 ± 2.87 vs. 3.90 ± 0.61 IU/l, $p = 0.33$, and testosterone 165 ± 138 vs. 201 ± 104 ng/dl, $p = 0.42$). During follow-up, clinical assessment of patients with hypopituitarism performed by the same physician revealed changes in the genital phenotype in 3 cases. Patient No. 9, aged 15 days, had micropenis with scrotal testes and, by the age of 2 months, had developed bilateral cryptorchidism; patient No. 12, aged 1 month, had both testes in the scrotum measuring 1 ml each, developed unilateral cryptorchidism at 4.5 months, and at 13 months of age, the testes had ascended to a nonpalpable position; patient No. 13 had unilateral microorchidism at 2.5 months and bilateral microorchidism at 4 months of age.

Girls

Congenital hypopituitarism was confirmed in 13 out of 19 girls (68%). Referral was mainly based on neonatal hypoglycemia and/or cholestasis. Five patients were referred due to at least one of the following causes: diabetes insipidus, septo-optic dysplasia or familial CPHD. As presumed, there were no anomalies in their external genitalia. One girl was lost to follow-up and another was excluded from the analysis due to incomplete biochemical data. Assessment of the gonadal axis in hypopituitary girls performed at a median age of 5 months (range 0.6–24) revealed normal FSH concentrations (mean 5.7 ± 2.7 IU/l), with undetectable estradiol in 7 of them. The other 5 girls had an FSH level below the normal range (0.1 ± 0.0 IU/l), and estradiol assayed in 2 patients resulted in one undetectable and one unexpectedly elevated level. Normal FSH and estradiol levels were observed in all patients with normal pituitary function (table 2; fig. 2).

Brain MRI identified anatomical abnormalities in the hypothalamic and pituitary regions in all patients (boys and girls) with CPHD (n = 34). These abnormalities were: anterior pituitary hypoplasia or agenesis, disrupted pituitary stalk, and ectopic or absence of a neurohypophysis signal. Midline defects were observed in a few patients.

Isolated Hypocortisolism

Six patients referred due to cholestasis and/or hypoglycemia had isolated hypocortisolism (5 boys). All had normal external genitalia as well as normal pituitary-gonadal axis function. Permanent isolated ACTH deficiency was confirmed in only 1 patient (No. 18) who showed a normal MRI and a normal sequencing of the *TBX19* gene. The remaining 5 patients were reevaluated after the age of 6 months, and all of them showed normal pituitary-adrenal function, confirming the presence of a transient disorder [20].

Discussion

In the present study, we showed that genital anomalies in boys with suspected hypopituitarism were highly predictive of an altered pattern of gonadotropin secretion during the postnatal GnRH-gonadotropin surge. Pituitary insufficiency in the first months of age is a life-threatening disorder, usually evidenced by nonspecific signs such as hypoglycemia, cholestasis or poor feeding due to sleepiness. In this context, genital anomalies in boys might lead to the diagnosis of congenital hypopituitarism. CHH is rare as an isolated disorder; it occurs more frequently in patients with other pituitary hormone deficiencies. Fetal gonadotropin deficiency in boys alters the normal development of the external genitalia to a variable extent, probably according to the timing and/or magnitude of the gonadotropin deficiency. Thus, a thorough clinical examination constitutes an invaluable tool for the identification of the disease. Furthermore, in patients with delayed puberty, the presence of micropenis and/or cryptorchidism indicates the absence of the postnatal physiological gonadotropic surge and reinforces the suspicion of hypogonadism [21]. However, it is worth noting that intrauterine isolated GH deficiency may also be associated with micropenis [22, 23]. Interestingly, we observed a progression of external genitalia towards a worsened phenotype in the first months of life in some patients. Similar findings have already been described in 3 boys with CHH; one of them even presented normal genitalia at birth [24]. This highlights the importance of gonadotropin not only in fetal life but also in the early postnatal period. Furthermore, delayed gonadotropin deficiency might also develop in patients with CPHD, and the underlying mechanism could be the gradual destruction of a pool of progenitor pituitary cells [25]. The latter raises the question as to whether boys with CPHD and normal external genitalia at birth might still be at risk of suffering from CHH.

Serum LH and testosterone surge is the most evident sign of postnatal GnRH activation in boys [3]; therefore, repeatedly low concentrations of these two hormones or an absent rise of LH in an intravenous GnRH test are the biochemical hallmarks of hypogonadotropic hypogonadism in boys under 6 months of age. This pattern reflects the existence of Leydig cell dysfunction, while Sertoli cell function appears to be less affected. A possible explanation is that the Sertoli cell population is not entirely dependent on gonadotropin secretion concomitant with the fact that the serum levels of FSH are less affected when compared to LH serum concentrations [26]. Hence, we emphasize that LH and testosterone in boys are the most important serum markers used to exclude CHH [3]. It is important to highlight the timing of the basal LH and testosterone measurements, as physiologically the concentrations are highest at 1–2 months of age and then decline to prepubertal concentrations by 6 months of age.

In the present study and in line with a previous report [27], CPHD appears to be more frequent in boys, even though in most patients, genital anomalies were undetected by pediatricians before their referral to us. Thus, the different prevalence between genders is apparently not related to the presence of genital anomalies.

Since the gonadotropic axis is not studied in a timely manner in patients with CPHD, pharmacological functional tests are then needed at an older age when the hypothalamic-pituitary-gonadal axis is in a quiescent period. However, the lack of normative values for stimulation tests in this period usually delays the diagnosis until puberty. It is well known that hypogonadism is an important cause of emotional distress in adolescents and also triggers low bone mineral density. The diagnosis of an altered gonadotropin function prior to pubertal age allows the prescription of gonadal steroid substitution [28–30] at the adequate chronological age, thus relieving emotional distress, while optimizing bone health and improving the quality of life. In addition, reduced fertility in hypogonadotropic patients is a cause of worry in adulthood. In boys with CHH, the treatment with recombinant gonadotropins during the postnatal GnRH surge period aims to mimic its physiological development and potentially improves future fertility by inducing testicular growth. Up

to date, studies of 3 boys aged 2, 3 and 8 months who had received treatment have been published [5, 12]. All of them responded with enlarged testicular volume and increased penile length. Nevertheless, there are no feasible conclusions about its effectiveness yet.

In girls, the absence of CHH clinical signs makes it more difficult to clinically identify the CHH group. However, abnormally low FSH concentrations might be a valuable tool to identify these patients. Measurements of serum estradiol are of little value, probably due to the poor sensitivity of the routine assay commonly used. To our knowledge, there are no reports on treating girls during the first 2 years of age to improve their future fertility.

To summarize, in patients with congenital hypopituitarism, the absence of the postnatal GnRH-gonadotropin surge is highly suggestive of a subjacent gonadal axis dysfunction. Evaluation of the clinical genital phenotype together with the assessment of serum pituitary and gonadal hormones throughout this period of life helps to corroborate the diagnosis of CHH and avoids the use of pharmacological functional tests to evaluate the gonadal axis in infant boys. These findings lead to optimize the identification of those patients eligible for an early gonadotropin treatment during infancy, the timing of hormone replacement therapy at the appropriate pubertal age and the approach for genetic analysis. In girls, however, the diagnosis of CHH in infancy probably requires validation with functional tests.

The proposed diagnosis of CHH in congenital multiple hypopituitary patients during the postnatal gonadotropic surge needs to be validated with reassessment of the gonadal function at pubertal age.

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