CLINICAL STUDY

Central hypocortisolism as part of combined pituitary hormone deficiency due to mutations of *PROP-1* gene

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Abstract

Background: One of the causes of combined pituitary hormone deficiency (CPHD) is represented by Prophet of Pit-1 (*PROP-1*) gene inactivating mutations. This disorder is generally characterized by GH, TSH, prolactin (PRL), and gonadotropin deficiency, but recent papers have described a concomitant alteration of the corticotrope function.

Objective: To make a detailed investigation of the hypothalamic–pituitary–adrenal axis in two sisters with *PROP-1* gene mutations.

Patients: Two female siblings (17 and 16 years old) with CPHD, belonging to a Brazilian family of consanguineous parents, presented with growth retardation and central hypothyroidism during childhood, and showed central hypogonadism at the age of puberty. No clear clinical symptoms and signs of hypocortisolism were present.

Methods: GH, TSH, free thyroxine, total tri-iodothyronine, PRL, LH, FSH, ACTH and cortisol were measured in basal condition and after appropriate testing. The molecular study was performed by PCR amplification and sequencing analysis of *PROP-1* gene.

Results: Both patients showed GH, PRL, LH and FSH deficiencies, associated with absent responses to an insulin tolerance test (ITT), TRH and GnRH injection. Circulating concentrations of TSH were normal in basal conditions, but failed to respond to a TRH test. Plasma ACTH concentrations were normal, but serum cortisol concentrations were below the lower limit of the normal range, showing a trend to decrease during 6 years of follow-up. The serum ACTH response to ITT was impaired, whereas its response to CRH was normal and prolonged. The cortisol response to both tests, and to the ACTH test, was clearly impaired. In both sisters, the genetic analysis showed the presence of a homozygous 2-bp deletion (296delGA) of *PROP-1* gene, which results in the synthesis of a protein with no residual functional activity.

Conclusion: In addition to GH, TSH, PRL and gonadotropin deficiency, patients with *PROP-1* gene mutations can present with late-onset central hypocortisolism, possibly because of the lack of important paracrine factors normally produced by the cells surrounding the corticotropes and absent in the pituitary of these patients, or because of progressive corticotrope apoptosis. This finding indicates the need for life-long endocrine monitoring of *PROP-1*-deficient patients.

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Introduction

Combined pituitary hormone deficiency (CPHD) is a rare disorder that results from an impaired pituitary function of varied etiopathogenesis. CPHD is characterized by impaired production of growth hormone (GH) and one or more of the other anterior pituitary hormones. In general this condition occurs sporadically and is mainly caused by organic lesions or damage secondary to breech delivery. However, familial occurrence of CPHD has been reported in several cases and it is characterized by autosomal recessive, autosomal dominant, or X-linked recessive inheritance. At present,

two of the main pituitary transcription factors are implicated in the onset of this condition: the human homologue of the mouse Pit-1 (*POU1F1*) and the Prophet of Pit-1 (*PROP-1*). *POU1F1* is a tissue-specific transcription factor (1–4), expression of which is necessary for the determination and differentiation of somatotropes, lactotropes and caudomedial thyrotropes (5, 6). *PROP-1* is a paired-like homeodomain protein expressed early in the pituitary gland. It is necessary for *POU1F1* gene expression, for the differentiation of the *POU1F1*-dependent cell lineages, and for the differentiation of the gonadotropes (7, 8). As a consequence, in humans, dominant and recessive mutations in the

POU1F1 gene cause CPHD with deficiency of GH, prolactin (PRL) and thyroid-stimulating hormone (TSH) (9–14). Affected patients show intact corticotropin and gonadotropin function, consistent with the presence of spontaneous puberty and fertility. In contrast, mutations of the *PROP-1* gene are responsible for the absence of the Pit-1-dependent cell lineages and for reduced numbers of gonadotropes, which result in the deficiency of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in addition to GH, PRL and TSH (15–25).

Here, we report on two Brazilian sisters with CPHD caused by a homozygous mutation of the *PROP-1* gene, thus confirming the importance of this transcription factor in the determination and function of multiple pituitary cell lineages. Moreover, we demonstrate that the clinical phenotype of CPHD caused by *PROP-1* gene mutations also encompasses corticotrope deficiency, which may lead to central hypoadrenalism in later life.

Participants

Informed consent from each family member and approval by the Hospital Ethics Committee were obtained before the study.

The patients are two female siblings with CPHD, aged 17 years (patient 1) and 16 years (patient 2), belonging to a Brazilian family of consanguineous parents. A third sister, aged 18 years, and the parents are in good health, with height and weight within the normal range for the Brazilian population. No familial history of delayed puberty is present. The affected sisters were born at term after uneventful pregnancies. The delivery was by cesarean section in patient 1 and by spontaneous delivery in patient 2. Birth weight and length were within normal standards. Neuromotor development was normal.

Growth retardation was noted by the mother when the older sister was 3 years old and the younger sister was 2 years old. However, the patients were referred to the clinician only at the ages of 7 and 6 years respectively, when symptoms of hypothyroidism, such as constipation, cold intolerance and fatigue became apparent. At that time, GH, TSH and PRL deficiency were first diagnosed. Unfortunately, it was not possible to investigate the patients further and to establish the hormonal replacement therapy with recombinant human (rh) GH and L-thyroxine (L-T₄) until the age of 11 and 10 years respectively, because of other serious problems in the family.

Before treatment with rhGH and L-T₄, height-SD scores (H-SDS) measured at 11 years in patient 1 and 10 years in patient 2, were –6.0 H-SDS and –4.9 H-SDS respectively. After 5 years of hormonal replacement therapy, H-SDS improved to –1.6 H-SDS and –2.0 H-SDS respectively, with augmentation of growth velocity (9.1 ± 4 and 9.0 ± 3.3 cm/year during 5 years of follow-up respectively). Now, both patients are of normal stature compared with those of the parents (152 cm in patient 1 and 149 cm in patient 2; mother's

and father's heights: 151 cm and 168 cm respectively) and normal weight and body mass index (BMI) (47.8 and 45.7 kg and 20.8 and 20.6 kg/m² in patients 1 and 2 respectively). However, bone age remains delayed in both patients, being of about 13 years, probably because of the concomitant presence of central hypogonadism which is responsible for severe delayed puberty in both sisters; at the ages of 16 and 15 years respectively, they showed spontaneous breast stage Tanner II and pubic hair stage Tanner I. This condition required estrogens and progesterone replacement therapy. To date, no clear clinical symptoms and signs of central hypocortisolism are present. Magnetic resonance imaging of the skull showed normal pituitary size (4.8 and 5.1 mm in patient 1s and 2, respectively compared with 5.2 ± 0.7 mm of pituitary height in matched controls) and morphology, normal stalk and normally located posterior pituitary lobe.

All the biochemical examinations performed in the patients were carried out when they were 16 and 15 years old respectively. Before measurement of TSH, PRL, LH and FSH concentrations, the replacement therapy with rhGH, L-T₄, estrogens and progesterone was discontinued for 2 months; GH, ACTH and cortisol were evaluated during the period of treatment.

Methods

Biochemical studies

Basal circulating concentrations of GH, TSH, free thyroxine (FT₄), total tri-iodothyronine (TT₃), PRL, LH and FSH were measured by an immunofluorimetric (IFMA) assay, using AutoDelfia technology (Wallac, Turku, Finland). Plasma ACTH and serum cortisol levels were measured on unextracted samples by IRMA (Nichols Institute, San Juan Capistrano, CA, USA) and RIA (Diagnostic products, Los Angeles, CA, USA) respectively. The intra- and interassay coefficients of variation were 3.1–7.3% and 4.6–5.4%, respectively. The lower limits of sensitivity were 0.45 pmol/l for plasma ACTH and 10 nmol/l for serum cortisol. Serum transcortin concentrations were evaluated by IRMA (Bouty, Cinisello-Balsamo, Italy).

The TSH and PRL responses to TRH (200 µg i.v.), and the LH and FSH responses to GnRH (100 µg i.v.) were evaluated after withdrawal of rhGH, L-T₄, estrogens and progesterone. In contrast, the cortisol response to ACTH (250 µg i.v. in a single administration and 250 µg i.m. every 24 h for 3 days) and CRH (1 µg/kg i.v.), and the GH, cortisol and ACTH responses to an insulin tolerance test (ITT: 0.15 U/kg i.v.) were evaluated during replacement therapy.

Genetic analysis of the *PROP-1* gene

The genetic analysis was performed by extracting genomic DNA from peripheral blood lymphocytes

Table 1 Serum TSH, PRL and gonadotropin concentrations in patients 1 (16 years old) and 2 (15 years old), in basal conditions and after stimulatory tests, as studied after 2 months of withdrawal of substitutive therapy.

Hormone	Patients or controls	Test			
		TRH		GnRH	
		Basal	Peak	Basal	Peak
TSH (mU/l)	1	1.8	0.7		
	2	2.6	0.4		
	controls	0.26–5	≥ 6		
PRL (mU/l)	1	92	92		
	2	120	17		
	controls	> 125	≥ 500		
LH (U/l)	1			0.2	0
	2			0.1	0.3
	controls†			> 3	> 12
FSH (U/l)	1			0.2	0
	2			0.2	0
	controls†			> 2	> 6

† Controls (*n* = 63) were matched for sex and age.

using standard methods. The coding sequence of the *PROP-1* gene was PCR-amplified with specific primers (sense prime: 5'-CGA ACA TTC AGA GAC AGA GTC CCA GA-3'; anti-sense primer: 5'-GAA TTC ACC ATG ATC TCC CA-3') to generate a 3.5 kb fragment. The PCR reaction was performed using 500 mg genomic DNA as template in a volume of 50 µl. The PCR cycles consisted of 30 s at 98 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 56 °C and 4 min at 68 °C. The PCR products were purified by gel electrophoresis followed by agarose gel DNA extraction. Direct sequencing of the double-stranded PCR fragments was performed by an automated method (PE Applied Biosystems, ABI PRISM 310 DNA Sequencer, Perkin-Elmer) using specific primers.

Results

Biochemical features

Both affected sisters exhibited GH, PRL, LH and FSH deficiencies, associated with absent response of these hormones to provocative stimuli (Tables 1 and 2). Circulating concentrations of TSH were normal in basal conditions, but failed to respond to TRH (Table 1). Without L-T₄ replacement therapy, basal FT₄ concentrations were below the normal range (2.5 and 3.9 pmol/l in patients 1 and 2 respectively; normal range 9–20 pmol/l). During L-T₄ treatment, circulating concentrations of FT₄ and TT₃ were in the normal range (FT₄ 15.2 and 15.7 pmol/l and TT₃ 1.5 and 1.7 nmol/l in patients 1 and 2 respectively; normal range 1.4–2.9 nmol/l). In basal conditions, circulating concentrations of ACTH were normal (Table 2), but serum cortisol concentrations were low in both sisters, showing a trend to decrease during 6 years of follow-up (Fig. 1). Serum transcortin concentrations were within the normal range (648 and 722 nmol/l in patients 1 and 2 respectively; normal range 620–740 nmol/l).

The serum ACTH response to ITT was impaired, but its response to CRH was normal and prolonged, as the peak response was observed at 120 min, whereas in normal subjects it occurs at 30 min (Table 2). The cortisol response to both tests (ITT and CRH), and to ACTH (250 µg i.v.) given in a single administration, was clearly impaired (Table 2). Nevertheless, a prolonged administration of ACTH (250 µg i.m. every 24 h for 3 days), performed at the patients' current ages of 17 and 16 years respectively, induced a normal cortisol increase in both of them (basal cortisol concentrations 66 and 71 nmol/l in patients 1 and 2 respectively; concentrations after 72 h 849 and 825 nmol/l in patients 1 and 2 respectively; normal response > 600 nmol/l).

Table 2 Serum cortisol, ACTH and GH concentrations in patients 1 (16 year old) and 2 (15 years old), in basal conditions and after stimulatory tests, during hormonal replacement therapy.

Hormone	Patient or controls†	Test								
		ITT			CRH			ACTH		
		Basal	Peak	Δ‡	Basal	Peak	Δ	Basal	Peak	Δ
Cortisol (nmol/l)	1	118	237	119	118	278	160	107	298	191
	2	314	281	0	237	287	50	198	356	158
	controls	140–700	≥ 500	≥ 200	140–700	≥ 550	≥ 200	140–700	≥ 500	≥ 200
ACTH (pmol/l)	1	5.7		6.3	4.2		13.5§			
	2	7.1		2.7	5.1		15.3§			
	controls	0.7–13.2		≥ 22	0.7–13.2		≥ 4.4			
GH (mU/l)	1	0.2		0						
	2	1.2		0						
	controls	< 4		≥ 20						

† Controls (*n* = 56) were matched for sex and age.

‡ Δ refers to the difference between the peak response and the basal value.

§ These values were obtained 120 min after CRH injection in the patients, and at 30 min after the injection in normal individuals.

Figure 1 Basal serum cortisol concentrations evaluated during 5 years of follow-up in the two patients. Blood samples for cortisol measurements were collected at 0800–0900 h. The horizontal dotted line represents the lowest limit of the normal range.

Genetic studies

Sequence analysis revealed in these affected sisters the presence of a homozygous 2-bp deletion at position 296 (296delGA) on exon 2 of the *PROP-1* gene. This deletion results in a frame shift that leads to a transitional stop signal at codon 109 (S109X). The predicted protein is truncated at residue 108 and lacks part of the homeo-domain, thus becoming unable to bind the promoter sites and to activate transcription of target genes (15). The parents and the third sister were heterozygous for this mutation.

Discussion

In this study, we described two Brazilian sisters affected with CPHD caused by *PROP-1* deficiency. In both patients, in agreement with previously reported data (20), the initial clinical sign of the disease was growth retardation. Height velocity was dramatically slow during childhood. However, the diagnosis of CPHD was not made until the sisters were aged 7 and 6 years, when symptoms of TSH deficiency became evident. At that time also, PRL deficiency was detected. A few years later, hormonal replacement therapy with rhGH and $L-T_4$ was established, leading to normalization of the final height. Both sisters now present normal stature, weight and BMI, but delayed bone maturation is still present. This finding may also be related to the concomitant hypogonadotropic hypogonadism documented by severe delayed puberty and lack of gonadotropin response to GnRH. At ages 16 and 15 years, biochemical studies in both patients showed very low basal GH, PRL, LH and FSH concentrations and complete absence of response to specific releasing

hormones. Without $L-T_4$ therapy, circulating concentrations of TSH at baseline were normal, but thyroid hormone concentrations were in the hypothyroid range, suggesting a central hypothyroidism resulting from the secretion of TSH molecules with reduced TSH bioactivity (26). Nevertheless, contrary to what is observed in central hypothyroidism of hypothalamic origin, which also may result in the secretion of bioinactive TSH, the TSH response to TRH was neither exaggerated or prolonged, but absent, in keeping with an impairment of thyrotrope development.

The most interesting finding of the present study relates to the adrenal function of our patients. It was observed that basal cortisol concentrations gradually declined over time and were clearly within the hypoadrenal range in both patients. In order to document the origin of such a hypocortisolism, an extensive biochemical analysis of the hypothalamic–pituitary–adrenal axis was carried out by evaluating serum cortisol and ACTH concentrations in basal conditions and after several provocative tests. The results showed a central origin of the hypocortisolism. Indeed, in both patients, cortisol concentrations failed to respond to all the stimulatory tests applied (ITT, CRH and ACTH given in one *bolus*). Moreover, circulating concentrations of ACTH, normal in basal conditions, failed to respond to ITT and showed a prolonged response to CRH. To further confirm the normal function of the adrenal glands, ACTH was given to the patients every 24 h and cortisol was measured after 24, 48 and 72 h, showing a normal increase.

This condition of central hypocortisolism seems to be of late onset and becomes evident gradually over time. Similar results have been reported recently by Pernasetti *et al.* (25), who found a tendency to ACTH/cortisol deficiency with age in six of 10 *PROP-1*-deficient patients from Brazil. Moreover, these authors noted that ACTH and cortisol responses to ITT and CRH exhibited a negative correlation with age. It is tempting to speculate that the progressive hypocortisolism may be related to the lack of important paracrine factors normally produced by the cells surrounding the corticotropes, which are absent from the pituitaries of these patients. Alternatively, it could reflect corticotrope apoptosis occurring over time in *PROP-1*-deficient patients (25).

Despite the presence of an impaired adrenal function, neither of these affected sisters presented clear symptoms or signs of hypocortisolism. However, we decided to treat them with cortisol replacement therapy in order to prevent the life-threatening features of adrenal insufficiency that may become apparent in the event of severe physical and psychological conditions of stress.

Although we found a normal pituitary gland in both patients at the ages of 16 and 15 years respectively, changes in pituitary size and morphology can occur over time in *PROP-1*-deficient patients. Mendonca *et al.*

(23) described one patient with PROP-1 deficiency who presented an enlarged and hyperintense pituitary gland at the age of 8.8 years and a hypoplastic anterior pituitary lobe 6 years later. Therefore, repeated imaging studies of the skull appear to be necessary in order to monitor the size of the pituitary in these patients.

Finally, genetic analysis showed a homozygous 2-bp deletion (296delGA) of the *PROP-1* gene in both patients. The third sister and the parents were heterozygous, suggesting an autosomal recessive pattern of inheritance of the disease. This deletion, which has been referred to as 301–302delAG by some authors (15, 18, 23–25), leads to a truncated protein at residue 108 that lacks the DNA-binding and the C-terminal *trans*-activation domains. Thus, when the 296delGA mutant is expressed in the context of mouse Prop-1, it lacks promoter binding and transcriptional activation activities (15). Until now, the same mutation has been noted in families of different origin, suggesting that it could represent a mutational 'hot spot' (18, 19). In fact, it is located in a sequence of three-GA repeats in exon 2 (296GAGAGAG) and it is a common opinion that repeat sequences show more susceptibility to mutations responsible for human diseases. Moreover, as functional studies have demonstrated that the 296delGA mutant has no residual functional activity, this mutation could account for a severe phenotype of CPHD, characterized by deficiency in all the anterior pituitary hormones. In fact, all the patients reported to date with PROP-1 deficiency and ACTH/cortisol impaired secretion, in addition to GH, TSH, PRL and gonadotropin deficiency, show the same genotype (i.e. 296delGA) (23–25). However, further studies are necessary to confirm this particular genotype/phenotype correlation.

In summary, the present study confirms that, in addition to GH, PRL, TSH and gonadotropin deficiency, patients with *PROP-1* gene mutations can present with late-onset cortisol insufficiency, possibly because of the lack of important paracrine factors normally produced by the cells surrounding the corticotropes and absent in the pituitary of these patients, or because of progressive corticotrope apoptosis. This distinct phenotype, characterized by the impaired secretion of all the anterior pituitary hormones, could be associated with the 296delGA mutation of *PROP-1* gene, as it represents a complete loss of function mutation. As the hypocortisolism seems to become more and more evident with advancing age in PROP-1-deficient patients, a complete endocrine evaluation and life-long monitoring of these patients are recommended in order to guarantee that they receive the potentially life-saving cortisol replacement therapy.

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