HORMONES 2013, 12(1):128-134

Case report

PROP-1 gene mutations in a 63-year-old woman presenting with osteoporosis and hyperlipidaemia

Maria Andrikoula,[1] Amalia Sertedaki,[2] Sofia Andrikoula,[3] Catherine Dacou-Voutetakis,[2] Agathocles Tsatsoulis[1]

[1]Department of Endocrinology, University Hospital of Ioannina, Ioannina, [2]Molecular Endocrinology Unit, First Department of Paediatrics, Athens University Medical School, Athens, [3]Department of Orthopaedics, University Hospital of Ioannina, Ioannina, Greece

ABSTRACT

***PROP-1* gene mutations have been reported as a cause of combined pituitary hormone deficiency. Physical and hormonal phenotypes of affected individuals are variable. We report a 63-year-old female who presented with osteoporosis. She was short, did not enter puberty spontaneously and had primary amenorrhea. Biochemical evaluation revealed secondary hypothyroidism and mixed hyperlipidaemia, while dynamic testing of pituitary function was diagnostic of hypopituitarism. Bone density in the lumbar spine disclosed osteoporosis. DNA analysis showed that the patient was homozygote for the R73H mutation of the *PROP-1* gene. The unfavourable long-term course of an untreated patient with *PROP-1* gene mutation emphasizes the need for early aetiologic classification and proper management and follow-up of patients with short stature and/or disturbances of pubertal development.**

Key words: ACTH deficiency, GH deficiency, Hypopituitarism, Osteoporosis

INTRODUCTION

Combined pituitary hormone deficiency (CPHD) is diagnosed when the production of two or more pituitary hormones is insufficient or absent. It can result either from birth trauma or asphyxia or from defects of genes controlling pituitary cell differentiation. It is mainly sporadic;[1] however, familial forms have also been described with autosomal recessive, autosomal dominant or X-linked recessive modes of inheritance.[2-4] Several developmental genes (*PIT-1*, *PROP-1*, *LHX-3*, *LHX-4*, *HESX-1, SIX-6, OTX-2, PTX-2, GLI-2* and *SOX-3*) have been identified as important for organ commitment and cell differentiation and proliferation and have been implicated in hypopituitarism in mice and humans.[5-8]

Prophet of Pit-1 (*PROP-1*) gene mutations as a cause of CPHD in humans was first recognised in 1998.[9] The hormonal deficiencies in *PROP-1* gene defects include growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), luteneizing hormone (LH) and follicle-stimulating hormone (FSH). In a subset of these patients, adrenocorticotropin hormone (ACTH) insufficiency develops as a

Address for correspondence:
Dr Maria Andrikoula, MD, PhD, 68 Dim. Hatzi Str., Ioannina, GR-45445, Greece, Tel: ++306944475080, Fax: ++302641100400, e-mail: mandrikoula@doctors.org.uk

Received 02-02-12, Accepted 07-05-12

relatively late manifestation, which thus far remains unexplained since the corticotroph cell is outside the lineage of cells affected by the *PROP-1* gene.[10] In addition, low values of dehydroepiandrosterone sulphate (DHEA-S) have been reported in the presence of normal pituitary-adrenal axis.[11]

To date, at least 24 distinct mutations of the *PROP-1* gene have been described, either homozygous recessive or compound heterozygous.[9,12-14] These mutations represent the most frequently reported aetiology of genetically determined CPHD.[13,15-17]

As previously mentioned, patients with *PROP-1* gene mutations can present with late-onset central hypocortisolism, possibly because important paracrine factors normally produced by the cells surrounding the corticotropes are absent in the pituitary of these patients, leading to progressive corticotrope cell apoptosis.[15,18,19] The physical and hormonal phenotypes of affected individuals vary widely, both within and between pedigrees, and this is often the reason for a delayed diagnosis. Pituitary morphology in patients with *PROP-1* gene mutations is variable. Normal, small or enlarged pituitary gland have all been reported. Although a small pituitary gland is frequently observed, especially in older subjects, a significant number of young patients with *PROP-1* gene mutations demonstrate pituitary enlargement.[19-21]

In this paper, we report a 63-year-old female who presented with osteoporosis and hyperlipidaemia and was very short (125cm). She was found to have central hypothyroidism, low GH, low cortisol, prolactin and gonadotropins which were caused by a homozygous mutation of the *PROP-1* gene. This case emphasizes the need for early aetiologic classification of short stature and long-term follow-up of patients with *PROP-1* mutations in order to detect and treat promptly impaired growth and pubertal development and, importantly, the late onset of corticotropin deficiency.

CASE REPORT

Case history

A 63-year-old female presented to the orthopaedic clinic for management of osteoporosis. Because of her extreme short stature and hyperlipidaemia refractory to three different pharmacologic agents, she was referred to the endocrine clinic for further investigation and treatment.

Physical examination showed that her height was 125 cm, the body weight 39 kg, the BMI 25 kg/m^2, the Breast development was Tanner Stage II and the Pubic Hair Tanner Stage II. The blood pressure was 140/60 mmHg without orthostatic hypotension. A history of absent puberty and primary amenorrhoea was reported; however, there was no history of disturbances in mental development. She mentioned having received estrogen treatment for a short period of time during her school years. Subsequently, treatment was discontinued on her own initiative and she was lost to follow-up. Her current medical status also included hypertension, hyperlipidaemia and osteoporosis, for which she was on relevant treatment with an angiotensin-converting enzyme inhibitor, pravastatin, ezetimibe, omega-3 fatty acids, risedronate and alphacalcidol. Her bone density, measured by DEXA, was diagnostic of osteoporosis in the lumbar spine [T-score (total): -3.4, Z-score (total): -1.44] and diagnostic of osteopenia in the hip [T-score (Total hip: -1.1, Neck: -1.8), Z-score (Total hip: 0.2, Neck: -0.2)] . She did not have any previous history of fractures. She was not aware of any family history of pituitary or thyroid disease; however, she mentioned that two of her female cousins (daughters of her mother's sister) also have short stature but have never been investigated further. As far as her parents were concerned, they were born in the same village, but there was no known consanguinity between them. Her three brothers are of normal height and have no hormonal deficiencies.

Methods

Informed consent from the patient and the approval of the Hospital Ethics Committee were obtained before initiating this study.

Hormonal evaluation

A blood sample for basal hormone levels was collected at 8.00 am following an overnight fasting.

Serum TSH, Free Thyroxine (FT4), Triiodothyronine (T3), PRL, LH, and FSH were determined using the automated chemiluminescence system (ACS 180, Bayer Diagnostics Europe Ltd., Dublin, Ireland).

A glucagon test was carried out following the intramuscular administration of glucagon (1 mg). Blood samples were obtained prior to and every 30 minutes for 3 hours for measurement of cortisol and GH. Adrenal function was also investigated by measuring cortisol levels prior to and 30 and 60 min following intravenous administration of tetracosactrin (synthetic ACTH, 250μg). GH and cortisol were determined by immunoradiometric assay (kit from CIS Bio-International, Gif-sur-Yvette, France) and the chemiluminescence immunoassay (Nichols Advantage, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA).

Genomic analysis of the PROP-1 gene

DNA was extracted from peripheral blood leucocytes employing the Maxwell 16 Instrument AS2000, automated purification system (Promega Corporation, Madison, USA). Exons 2 and 3 and their flanking intronic sequences of the *PROP-1* gene were PCR amplified and directly sequenced in the forward and reverse direction on an automated sequencer (ABI 3100 Avant, Applied Biosystems). PCR conditions and primers used for amplification and sequencing have been previously described.[19]

Results and clinical course

Laboratory data are shown in Tables 1 and 2. Serum levels of FT4 and T3 were very low with inappropriately normal levels of TSH, indicating central hypothyroidism and a possible additional cause for our patient's hyperlipidaemia. Creatinine kinase (CK) value was high, and 25-hydroxycholecalciferol levels ($25OHD_3$) were low.

FSH and LH values were low despite the patient's menopausal age. PRL levels were low and plasma ACTH level was at the lower limit of the normal range [8.9 pg/ml (NV: 7-52pg/ml)]. Peak growth hormone and cortisol values during glucagon test were <0.05ng/ml (NV ≥10ng/ml) and 11.6μg/dl (NV ≥18μg/dl), respectively. Plasma cortisol at 08.00 was low and 60 min post Synacthen was 16.2 μg/dl (NV ≥18 μg/dl). These results showed inadequate response of cortisol secretion to the administration of either glucagon or synthetic ACTH. Although the insulin tolerance test (ITT) is the most powerful test to evaluate the hypothalamic-pituitary-adrenal (HPA) axis, it was not performed as the patient did not give her consent.

Table 1. Laboratory findings upon admission of patient[1]

	Patient	Normal range
Fasting glucose, mg/dl	75	50-110
Urea, mg/dl	48	11-54
Creatinine, mg/dl	0.8	0.6-1.2
Sodium, mEq/l	136	135-153
Potassium, mEq/l	5.23	3.5-5.3
AST, IU/l	61	10-35
ALT, IU/l	31	10-35
γGT, IU/l	35	6-32
ALP, IU/l	59	30-125
Calcium, mg/dl	10	8.2-10.6
Phosphate, mg/dl	4.2	2.5-5
Total protein, mg/dl	8.1	6-8.4
Albumin, mg/dl	4.8	3.4-5
Total cholesterol, mg/dl	248	110-200
HDL-cholesterol, mg/dl	57	35-70
LDL-cholesterol, mg/dl	154	<130
Triglycerides, mg/dl	185	40-175
CK, IU/l	495	25-160
LDH, IU/l	446	115-230

[1]All values refer to serum concentrations.
AST: aspartate aminotransferase; ALT: alanine aminotransferase; γGT: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase; HDL: high density lipoprotein; LDL: low density lipoprotein; TGL: triglycerides; CK: creatinine kinase; LDH: lactate dehydrogenase.

Sequencing of exons 2 and 3 of the *PROP-1* gene revealed that the patient was homozygote for the previously described exon 2 mutation, p.R73H,[15] thus clarifying the aetiology of the patient's CPHD. Replacement therapy with hydrocortisone (10 mg am, 5 mg pm) was initiated followed by replacement therapy with L-thyroxine (50 μg/day) and $25OHD_3$ (1200 units/day). Her hypolipidaemic treatment was discontinued and lipid levels decreased (Total cholesterol: 173 mg/dl, high density lipoprotein (HDL): 43, triglycerides (TGL): 142 mg/dl). Under thyroxine treatment, circulating concentrations of FT4 and T3 stayed within the normal range [FT4 1.04 ng/dl (NV: 0.7-1.85 ng/dl) and T3 1.01 ng/ml (NV: 0.5-1.4 ng/ml)]. She has been doing well and feeling more

Table 2. Hormonal data of patient during / at admission[1]

	Patient	Normal range
TSH, μIU/ml	1.79	0.5–4.8
T3, ng/ml	0.37	0.5-1.4
FT4, ng/dl	0.4	0.5-4.8
Prolactin, ng/ml	2.7	5.2-26.5
LH, mIU/ml	0.1	Follicular phase <14
		Ovulation 20-70
		Luteal phase <16
		Postmenopause 20-70
FSH, mIU/ml	0.1	Follicular phase 2-10
		Ovulation 9-18
		Luteal phase <9
		Postmenopause 20
E2, pmol/l	37	Follicular phase 77-921
		Ovulation 140-2382
		Luteal phase 77-1145
		Postmenopause <529
Testosterone, ng/ml	0.1	0.3-1.4
SHBG, nmol/l	48.7	18-114
PTH, pg/ml	52.9	12-72
Basal serum DHEA-S, ng/ml	150	1500-5500
		Postmenopause 200-800
Synacthen test (following tetracosactrin 250 μg I.V)		
Basal serum ACTH, pg/ml	8.9	9–52
Basal serum cortisol, μg/dl	3.7	5–23
Peak serum cortisol, μg/dl, 60 min post Synacthen	16.2	peak >18
Glucagon test (following 1 mg glucagon i.m)		
Basal serum cortisol, μg/dl	5.2	5–23
Peak serum cortisol, μg/dl, 180 min post glucagon	11.6	peak >18
Basal serum growth hormone, ng/ml,	<0.05	
Serum growth hormone, ng/ml, throughout glucagon test	<0.05	peak >10

[1]All values refer to serum concentrations
E2: estradiol; FSH: follicle-stimulating hormone; LH: luteneizing hormone; PRL: prolactin; SHBG: sex-hormone binding globulin; DHEA-S: dehydroepiandrosterone sulphate; T3: triiodothyronine; TSH: thyroid-stimulating hormone; FT4: free thyroxine; PTH: parathyroid hormone; ACTH: adrenocorticotropin hormone.

energetic as a sense of general fatigue, which had not been mentioned, has improved. Unfortunately, MRI of the pituitary gland was not possible since the patient refused because of claustrophobia.

DISCUSSION

CPHD is a rare disorder characterized by impaired production of GH and one or more of the other anterior pituitary hormones. Two pituitary transcription factors have been mainly implicated in the pathogenesis of this condition: the human homologue of the mouse Pit-1 (*POU1F1*)[5,23] and *PROP-1.*[6,24] The *PROP-1* gene is necessary for *POU1F1* gene expression and consequently for the differentiation of the *POU1F1*-dependent cell lineages (somatotropes, lactotropes and caudomedial thyrotropes) as well as for the differentiation of the gonadotropes.[6,24] Consequently, mutations of the *PROP-1* gene are responsible for the absence of the *Pit1*-dependent cell lineages and in addition for reduced numbers of gonadotropes, resulting in the deficiency of GH, PRL, TSH, FSH and LH.[9,13,20,25-27]

The human *PROP-1* gene encodes a protein of 226 aminoacids and its mutations account for the majority of cases of recessively inherited CPHD in persons of European origin.[9,12,13] The first examples of *PROP-1* mutations in humans with CPHD were reported in 1998,[9] following the report of Sornson et al in 1996 of the Ames dwarf mice mutation.[6] Thus far, at least 24 distinct mutations of the *PROP-1* gene have been identified in over 170 patients. The most common types of mutation are a two-base-pair deletion in codon 101 (301-302delAG)[28] and a one-base-pair deletion in codon 50 (150delA). Several other missense, nonsense and splice site mutations have been described, such as p.R73C, p.R73H, p.F88S, p.F117I, p.R120C, p.R120H, p.R99X, p.Q83X.[9,13,15,21,22,26,29] Patients with *PROP-1* gene mutations exhibit some phenotypic variability, involving age of onset of hormone deficiencies,[30,31] pituitary size[9,28,31,32] and cortisol secretion.[9,28]

In a number of patients with *PROP-1* gene mutations, late-onset hypocortisolism has been detected,[15,18] with a tendency for ACTH and cortisol deficiencies to emerge with advancing age.[20,27,33] Such a progressive ACTH deficiency with age is not observed in Ames dwarf mice.[6] Moreover, it has not been linked to a

specific mutation. This suggests that a more complex mechanism than simple failure of embryonic development of the corticotroph cell lineage may be involved, as proposed by Pernasetti et al.[27]

In our patient with CPHD a p.R73H homozygous mutation in the 2nd exon of the *PROP-1* was identified. The patient remained undiagnosed and untreated till the age of 63 years, when she presented with osteoporosis and hyperlipidaemia refractory to medical treatment. Her bone density was diagnostic of osteroporosis in the lumbar spine and osteopenia in the hip, both due to hypogonadotropic hypogonadism and $25OHD_3$ deficiency. Her serum levels of FT4 and T3 were very low with inappropriately normal levels of TSH, indicating the diagnosis of central hypothyroidism and the cause of her hyperlipidaemia that was refractory to medical treatment. The levels of other pituitary hormones, such as PRL, FSH and LH, and the levels of DHEA-S were also low, confirming the diagnosis of anterior lobe pituitary insufficiency.[9] Her short stature, lack of pubertal development as well as primary amenorrhoea indicated that her pituitary insufficiency was manifested early in life. Dynamic pituitary testing with glucagon test revealed GH as well as ACTH deficiency. Although ITT remains the gold standard test for assessing the entire HPA axis, several authors advocate the Short Synacthen Test (SST) with appropriate cut-offs as the first line test.[34-36] While controversy exists about the cut-offs and diagnostic reliability, the potential for missing subtle or recent HPA defects remains a possibility.[37] Our patient had a longstanding hypopituitarism and additionally she declined to have an ITT, therefore we decided to perform a SST. Despite the SST result being consistent with partial secondary hypoadrenalism due to long-term ACTH insufficiency, this patient had never had any clinical or laboratory findings of hypoadrenalism, such as orthostatic hypotension, hyponatraemia or hypoglycaemia. This may be due to the well known fact that patients with incomplete hypocortisolism may experience severe symptoms only during periods of stress and remain undiagnosed for long periods. In view of the above, we decided to start cortisol replacement prior to thyroxine treatment in order to prevent the life-threatening features of adrenal insufficiency that may become apparent in the event of severe physical and psychological stress. Pituitary size of the patient is not known as she refused to have a pituitary MRI due to claustrophobia.

The mutation p.R73H of the *PROP-1* gene was first described in 2001 by Vallette-Kasic et al.[15] Five years later, there were two more patients found to have the same mutation without any family history of CPHD.[8] Our patient is apparently the oldest to be reported so far with CPHD due to the above mutation. Although she does not have any known family history of CPHD, she admitted having two cousins of similar age with short stature who have not been investigated.

In conclusion, we present a female patient with short stature, hyperlipidaemia refractory to medical treatment and osteoporosis who was diagnosed with hypopituitarism due to a *PROP-1* gene mutation at the age of 63. In addition to GH, PRL, TSH and gonadotropin deficiency, our patient also had partial cortisol insufficiency. Our case illustrates the importance of investigating patients with short stature to identify the hormonal and genetic cause and offer the most appropriate treatment. As the clinical phenotypes of human *PROP-1* mutations may be variable and modified by time, it is important to assure etiologic classification and long-term follow-up of such patients in order to improve quality of life as well as morbidity and mortality.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Rimoin DL, Merimee TJ, Rabinowitz D, McKusick VA, 1968 Genetic aspects of clinical endocrinology. Recent Prog Horm Res 24: 365-437.
2. Watkins-Chow DE, Camper SA, 1998 How many homeobox genes does it take to make a pituitary gland? Trends Genet 14: 284-290.
3. Procter AM, Phillips III JA, Cooper DN, 1998 The molecular genetics of growth hormone deficiency. Hum Genet 103: 255-272.
4. Lagerstrom-Fermer M, Sundvall M, Johnsen E, et al, 1997 X-Linked recessive panhypopituitarism associated with a regional duplication in Xq25–q26. Am J Hum Genet 60: 910-916.
5. Li S, Crenshaw III EB, Rawson EJ, Simmons DM,

Swanson LW, Rosenfeld MG, 1990 Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene Pit-1. Nature 347: 528-533.
6. Sornson MW, Wu W, Dasen JS, et al, 1996 Pituitary lineage determination by Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. Nature 384: 327-333.
7. Dattani MT, Martinez-Barbera JP, Thomas PQ, et al, 1998 Mutations in the homeobox gene HESX1/Hesx1 associated with septo-optic dysplasia in human and mouse. Nat Genet 19: 125-133.
8. Reynaud R, Gueydan M, Saveanu A, et al, 2006 Genetic screening of combined pituitary hormone deficiency: experience in 195 patients. J Clin Endocrinol Metab 91: 3329-3336.
9. Wu W, Cogan JD, Pfaffle RW, et al, 1998 Mutations in PROP1 cause familial combined pituitary hormone deficiency. Nat Genet 18: 147-149.
10. Voutetakis A, Sertedaki A, Livadas S, et al, 2004 Corticotroph failure in patients with PROP1 gene mutations is associated with variable pituitary morphology, clinical manifestations and ACTH response to CRH. Horm Res 62: Suppl 2: 21 Abstract book.
11. Voutetakis A, Livadas S, Sertedaki A, Maniati-Christidi M, Dacou-Voutetakis C, 2001 Insufficient adrenarche in patients with combined pituitary hormone deficiency caused by a PROP-1 gene defect. J Ped Endocrinol Metab 14: 1107-1111.
12. Cogan JD, Wu W, Phillips III JA, et al, 1998 The PROP1 2-base pair deletion is a common cause of combined pituitary hormone deficiency. J Clin Endocrinol Metab 83: 3346-3349.
13. Deladoey J, Fluck C, Buyukgebiz A, et al, 1999 'Hot spot' in the PROP1 gene responsible for combined pituitary hormone deficiency. J Clin Endocrinol Metab 84: 1645-1650.
14. Kelberman D, Dattani MT, 2007 Hypopituitarism oddities: congenital causes. Horm Res 68: Suppl 5: 138-144.
15. Vallette-Kasic S, Barlier A, Teinturier C, et al, 2001 PROP1 Gene Screening in Patients with Multiple Pituitary Hormone Deficiency Reveals Two Sites of Hypermutability and a High Incidence of Corticotroph Deficiency. J Clin Endocrinol Metab 86: 4529-4535.
16. Turton JP, Mehta A, Raza J, et al, 2005 Mutations within the transcription factor PROP1 are rare in a cohort of patients with sporadic combined pituitary hormone deficiency (CPHD). Clin Endocrinol 63: 10-18.
17. Rainbow LA, Rees SA, Shaikh MG, et al, 2005 Mutation analysis of POUF-1, PROP-1 and HESX-1 show low frequency of mutations in children with sporadic forms of combined pituitary hormone deficiency and septo-optic dysplasia. Clin Endocrinol (Oxf) 62: 163-168.
18. Asteria C, Oliveira JHA, Abucham J, Beck-Peccoz P, 2000 Central hypocortisolism as part of combined pituitary hormone deficiency due to mutations of PROP-1 gene. Eur J Endocrinol 143: 347-352.
19. Voutetakis A, Argyropoulou M, Sertedaki A, et al, 2004 Pituitary magnetic resonance imaging in 15 patients with PROP1 gene mutations: pituitary enlargement may originate from the intermediate lobe. J Clin Endocrinol Metab 89: 2200-2206.
20. Mendonca BB, Osorio MG, Latronico AC, Estefan V, Lo LS, Arnhold IJ, 1999 Longitudinal hormonal and pituitary imaging changes in two females with combined pituitary hormone deficiency due to deletion of A301, G302 in the PROP1 gene. J Clin Endocrinol Metabol 84: 942-945.
21. Fofanova O, Takamura N, Kinoshita E, et al, 2000 MR imaging of the pituitary gland in children and young adults with congenital combined pituitary hormone deficiency associated with *PROP1* mutations. Am J Roentgenol 174: 555-559.
22. Voutetakis A, Maniati-Christidi M, Kanaka-Gantenbein C, et al, 2004 Prolonged jaundice and hypothyroidism as the presenting symptoms in a neonate with a novel PROP1 gene mutation (Q83X). Eur J Endocrinol 150: 257-264.
23. Ingraham HA, Chen R, Mangalam HJ, et al, 1988 A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. Cell 50: 519-529.
24. Andersen B, Pearse II RV, Jenne K, et al, 1995 The Ames dwarf gene is required for Pit-1 gene activation. Dev Biol 172: 495-503.
25. Fluck C, Deladoey J, Rutishauser K, et al, 1998 Phenotypic variability in familial combined pituitary hormone deficiency caused by a PROP1 gene mutation resulting in the substitution of Arg3Cys at codon 120 (R120C). J Clin Endocrinol Metab 83: 3727-3734.
26. Duquesnoy P, Roy A, Dastot F, et al, 1998 Human Prop-1: cloning, mapping, genomic structure. FEBS Lett 437: 216-220.
27. Pernasetti F, Toledo SP, Vasilyev VV, et al, 2000 Impaired adrenocorticotropin-adrenal axis in combined pituitary hormone deficiency caused by a two-base pair deletion (301–302delAG) in the prophet of Pit-1 gene. J Clin Endocrinol Metab 85: 390-397.
28. Parks JS, Tenore A, Bongiovanni AM, Kirkland RT, 1978 Familial hypopituitarism with large sella turcica. N Engl J Med 298: 698-702.
29. Osorio MGF, Kopp P, Marui S, Iatronico AC, Mendonca BB, Arnhold IJ, 2000 Combined pituitary hormone deficiency caused by a novel mutation of a highly conserved residue (F88S) in the homeodomain of PROP-1. J Clin Endocrinol Metab 85: 2779-2785.
30. Fofanova O, Takamura N, Kinoshita E, Parks JS, Brown MR, Peterkova VA, 1998 Compound heterozygous deletion of the PROP-1 gene in children with combined pituitary hormone deficiency. J Clin Endocrinol Metab 83: 2601-2604.
31. Underwood LE, Radcliffe WB, Guinto FC, 1976 New standards for assessment of sella turcica volume in children. Pediatr Radiol 119: 651-654.
32. Radovick S, Nations M, Du Y, Berg LA, Weintraub

BD, Wondisford FE, 1992 A mutation in the POU-homeodomain of Pit-1 responsible for combined pituitary hormone deficiency. Science 257: 115-118.
33. Agarwal G, Bhatia V, Cook S, Thomas PQ, 2000 Adrenocorticotropin Deficiency in Combined Pituitary Hormone Deficiency Patients Homozygous for a Novel PROP1 Deletion. J Clin Endocrinol Metab 85: 4556-4561.
34. Stewart PM, Corrie J, Seckl JR, Edwards CR, Padfield PL, 1988 A rational approach for assessing the hypothalamo-pituitary-adrenal axis. Lancet 1: 1208-1210.
35. Clayton RN, 1996 Short Synacthen test versus insulin stress test for assessment of the hypothalamo-pituitary-adrenal axis: controversy revisited. Clin Endocrinol (Oxf) 44: 147-149.
36. Oelkers W, 1996 Adrenal insufficiency. N Engl J Med 335: 1206-1212.
37. Soule SG, Fahie-Wilson M, Tomlinson S, 1996 Failure of the short ACTH test to unequivocally diagnose long-standing symptomatic secondary hypoadrenalism. Clin Endocrinol (Oxf) 44: 137-140.