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High Prevalence of *PROP1* Gene Mutations in Hungarian Patients with Childhood-Onset Combined Anterior Pituitary Hormone Deficiency

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Combined pituitary hormone deficiency is characterized by the impaired production of pituitary hormones, commonly including growth hormone. The pathomechanism of the childhood-onset form of this disorder may involve germline mutations of genes encoding pituitary transcription factors, of which *PROP1* gene mutations have been studied most extensively. However, controversy exists about the significance of *PROP1* gene mutations, as both low and high frequencies have been reported in these patients. Because the different results may be related to differences in patient populations and/or the variability of clinical phenotypes, we performed the present study to examine the prevalence and spectrum of *PROP1* gene mutations in 35 patients with non-acquired childhood-onset growth hormone deficiency combined with at least one other anterior pituitary hormone deficiency. Genetic testing indicated the presence of disease-causing mutations in exons 2 and 3 of the *PROP1* gene in 15 patients (43% of all patients; homozygous mutations in 10 patients and compound heterozygous mutations in 5 patients). Comparison of clinical data of patients with and without *PROP1* gene mutations failed to show significant differences, except an earlier growth retardation detected in patients with *PROP1* gene mutations. In one patient with *PROP1* gene mutation, radiologic imaging showed an enlargement of the anterior lobe of the pituitary, whereas the other patients had hypoplastic or normal pituitary gland. All patients with *PROP1* gene mutations had normal posterior pituitary lobe by radiologic imaging. These results indicate that using our inclusion criteria for genetic testing, *PROP1* gene mutations can be detected in a high proportion of Hungarian patients with non-acquired childhood-onset growth hormone deficiency combined with at least one other anterior pituitary hormone defect.

Key Words: Combined pituitary hormone deficiency; hypopituitarism; *PROP1* gene; pituitary transcription factors.

Introduction

Combined pituitary hormone deficiency (CPHD) is characterized by two or more pituitary hormone defects. The disorder has an estimated incidence of approx 1 in 8000 births. It can be congenital or acquired, and both forms have a heterogeneous etiology *(1,2)*. Recently, several signaling processes and transcription factors have been identified, which play a crucial role in pituitary organogenesis, cell lineage proliferation, and differentiation *(3,4)*. These pituitary transcription factors have been implicated in the development of congenital CPHD and genetic testing in some studies has indicated the presence of disease-causing mutations as high as 50% of patients with familial CPHD *(5)*. Of the several pituitary transcription factors identified so far, *PROP1* (prophet of *Pit1*) and *Pit1* (pituitary transcription factor 1) gene mutations have been studied most extensively.

The *PROP1* gene is expressed early in embryonic development and, therefore, is crucial for the functional differentiation of pituitary somatotrophs, thyrotrophs, gonadotrophs, and lactotrophs. However, there is a great phenotypic variability among patients with *PROP1* gene mutations causing CPHD *(6,7)*. Growth hormone deficiency occurs most commonly, but growth retardation can be modest or severe. In addition, retrospective longitudinal data analysis of patients with *PROP1* gene mutations showed a progressive deterioration of anterior pituitary hormone secretion, including ACTH (corticotrophin) insufficiency *(8)*.

Despite several studies, there is a controversy about the prevalence of *PROP1* gene mutations in patients with con-

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Table 1
Patient Characteristics[a]

	Mother's height SDS	Father's height SDS	At the time of diagnosis of GHD: CA (yr)	BA–CA (yr)	Height (SDS)	Age at the time of diagnosis of other hormone deficiencies (yr): TSH	LH, FSH	ACTH
All patients (n = 35)	0.2 ± 1.2 (n = 23)	–0.1 ± 0.9 (n = 23)	7.7 ± 3.6 (n = 34)	–3.7 ± 2.0 (n = 25)	–3.8 ± 0.8 (n = 26)	7.7 ± 3.4 (n = 30)	17.8 ± 2.9 (n = 22)	14.1 ± 5.8 (n = 9)
Patients with *PROP1* gene mutation (n = 15)	0.7 ± 0.9 (n = 15)	0.1 ± 0.8 (n = 15)	6.3 ± 1.6* (n = 15)	–3.3 ± 1.8 (n = 14)	–3.8 ± 0.74 (n = 15)	6.7 ± 1.2 (n = 15)	17.9 ± 3.4 (n = 11)	9.3 ± 3.2 (n = 3)
Patients without *PROP1* gene mutation (n = 20)	–0.8 ± 1.0 (n = 8)	–0.6 ± 0.9 (n = 8)	8.9 ± 4.4 (n = 19)	–4.1 ± 2.2 (n = 11)	–3.9 ± 0.9 (n = 11)	8.6 ± 4.5 (n = 15)	17.8 ± 2.6 (n = 11)	16.5 ± 5.4 (n = 6)

[a]Height of parents, the age and height at the time of the diagnosis of growth hormone deficiency, and the age at the diagnosis of other pituitary hormone deficiencies in all patients with combined pituitary hormone deficiency and in those with and without mutations of the *PROP1* gene.

*$p < 0.05$; GHD, growth hormone deficiency; CA, chronological age; BA-CA, retardation of bone age; SDS, standard deviation score; TSH, thyroid-stimulating hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; ACTH, corticotrophin.

genital CPHD. In some studies *PROP1* gene mutations were absent or occurred rarely *(9–11)* whereas in other studies *PROP1* gene mutations accounted for the majority of cases with CPHD *(12)*. Interestingly, studies showing a high prevalence reported a few common mutations of the *PROP1* gene, which raised the question of whether these recurrent mutations are ethnic-specific *(10)*. It is also possible that the different frequencies of *PROP1* gene mutations observed in previous studies may be related to the great phenotypic variability of CPHD patients, which may influence patient selection for genetic testing. Therefore, in the present study we examined the prevalence and spectrum of *PROP1* gene mutations in 35 patients with non-acquired childhood-onset growth hormone deficiency combined with at least one other anterior pituitary hormone defect. The first Hungarian patient with CPHD caused by a homozygous 296-302delGA mutation of the *PROP1* gene has already been reported *(13)*.

Results

Of the 35 patients with childhood-onset CPHD, we identified 15 patients (43%) with disease-causing *PROP1* gene mutations. Of the 15 patients, 10 patients had homozygous mutations (296-302delGA mutation in exon 2 in four patients, 150delA mutation in exon 2 in four patients, C217T mutation in exon 2 in one patient, and F117I mutation in exon 3 in one patient), whereas compound heterozygous mutations were detected in five patients (150delA/296-302delGA mutation in three patients, 150delA/ F117I mutation in one patient, and R99X/296-302delGA mutation in one patient). These findings indicated that in our patients 80% of the mutant alleles was attributed to 150delA and 296-302delGA mutations, confirming the presence of a mutational "hot spot" already reported in previous studies.

All CPHD patients were born with normal birth weights and lengths (data are not shown). Parental height was also normal. Breech presentation and neonatal distress was recorded in eight patients. All patients were diagnosed with GH (growth hormone) deficiency based on decreased serum GH responses to insulin, arginine, and, in some cases, levodopa or clonidine stimulation with serum peak levels of GH less than 7 ng/mL on two independent tests. As shown in Table 1, all of our patients presented with a marked growth retardation at the time of diagnosis (mean age, 7.7 ± 3.6 yr, with a bone age retarded by 3.7 ± 2.0 yr on average and with a mean height of –3.8 ± 0.8 SDS). When these clinical parameters were separately analyzed in patients with and without *PROP1* gene mutations, the results indicated a significantly younger chronological age at the time of the diagnosis of GH deficiency in patients who had *PROP1* gene mutations compared to those who had negative genetic testing (6.3 ± 1.6 vs 8.9 ± 4.4 yr). By contrast, the chronological age at the time of the diagnosis of TSH (thyroid-stimulating hormone), LH (luteinizing hormone), and FSH (follicle-stimulating hormone) deficiencies and the prevalence of these hormone defects were similar in the two groups. ACTH deficiency was detected in three patients with *PROP1* gene mutations at the mean age of 9.3 ± 3.2 yr and in six patients without *PROP1* gene mutation at the age of 16.5 ± 5.4 yr, but the difference between the two groups was not significant (Table 1). Adrenal crisis was noted in one adult patient. Serum prolactin levels were low or normal in all patients (not shown in the Table).

TSH deficiency was diagnosed early in both groups of patients; it was detected at the time of the diagnosis of GH deficiency in all patients with *PROP1* gene mutations and in the majority of patients without *PROP1* gene mutations. All patients with *PROP1* gene mutations developed GH

Table 2
MRI Imaging in Patients with Combined Pituitary Hormone Deficiency

	Pituitary				
	Anterior lobe			Posterior lobe	
	Hypoplasia	Normal	Hyperplasia	Eutopic	Ectopic
All patients (n = 22)	11	10	1*	15	7
Patients with *PROP1* gene mutations (n = 7)	0	6	1*	7	0
Patients without *PROP1* gene mutations (n = 15)	11	4	0	8	7

*Regression within 6 mo.

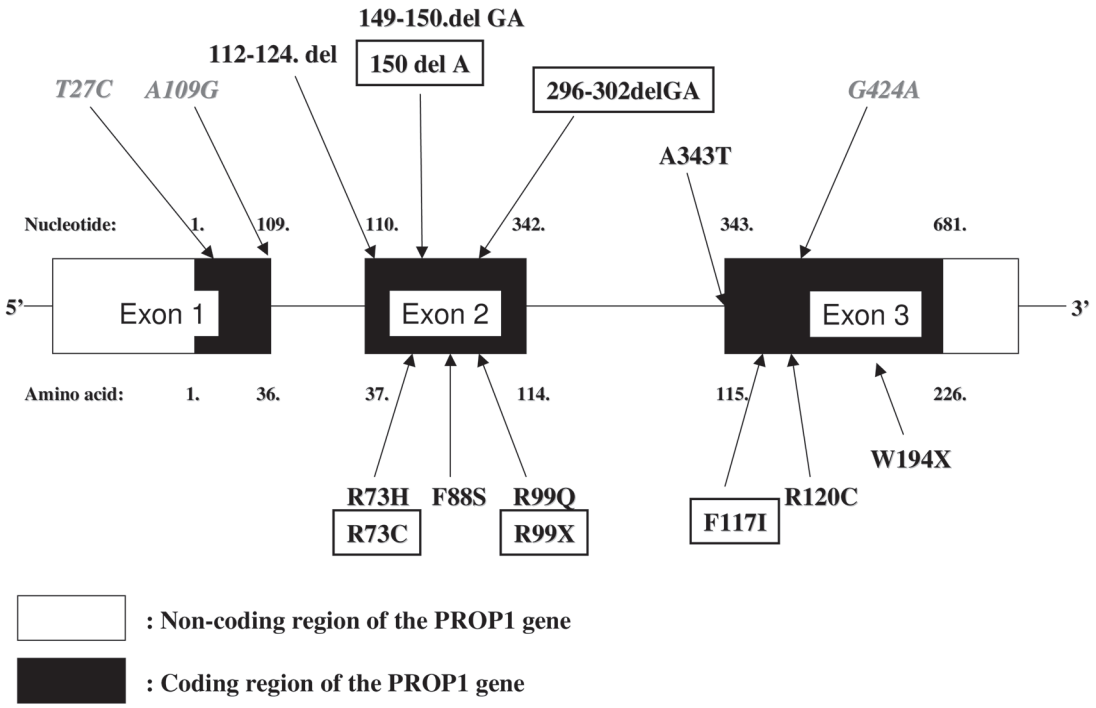

Fig. 1. Schematic illustration of the *PROP1* gene. Arrows indicate the location of reported mutations (bold letters) and polymorphisms (italics letters). All mutations detected in the present study (shown in boxes) have been already reported.

and TSH deficiencies by the age of 10 yr. By contrast, the prevalence of these hormone deficiencies in patients without *PROP1* gene mutations was lower at this age and was gradually increased until the age of 20 yr. In both groups of patients LH and FSH deficiencies were usually diagnosed after the expected pubertal age. ACTH deficiency was a relatively infrequent finding in both groups.

Table 2 summarizes the results of pituitary MRI imaging performed in 22 patients. One patient with *PROP1* gene mutation showed a transient enlargement of the anterior lobe of the pituitary presumably due to hyperplasia, whereas all other patients with or without *PROP1* gene mutations had a normal pituitary gland or pituitary hypoplasia. In all patients with *PROP1* gene who had pituitary MRI imaging the posterior lobe was normal. By contrast, pituitary MRI imaging showed an ectopic posterior lobe in 7 of the 15 patients without *PROP1* gene mutations.

Discussion

In 1996 Sornson and coworkers *(14)* reported the identification of *PROP1* gene in multiple pituitary-hormone-deficient Ames dwarf mice. Two years later the human homolog *PROP1* gene was cloned and mapped, and its genomic structure was identified *(15)*. The human gene is mapped to chromosome 5q, and it has three coding exons. The product of the *PROP1* gene is Prop1, a homeodomain transcription factor consisting of 226 amino acids. Prop1 is essential for the expression of Pit1 *(16)*. In 1998, Wu and coworkers *(17)* reported the first patients with familial CPHD who proved to have homozygous or compound heterozygous inactivating mutations of the *PROP1* gene. Until recently, at least 13 different mutations, including missense, frameshift, and splice site mutations, deletions, and insertions have been identified in exons 2 and 3, but none in exon 1 of the *PROP1* gene (Fig. 1). The three tandem repeats of the GA dinucle-

otides at location 296–302 represent the "hot-spot" region for mutation, and mutations involving this region cause preterm truncation of the homeodomain *(18,19)*.

PROP1 gene mutations cause reduced DNA binding and transcriptional activity of the protein product leading to growth hormone, thyroid stimulating hormone, prolactin, luteinizing hormone, and follicle-stimulating hormone deficiencies *(20)*. ACTH deficiency is not an obvious part of the clinical phenotype but it manifests rather frequently in adulthood *(21,22)*. It appears that *PROP1* gene does not play a role in the differentiation and hormone production of corticotrophs, but other mechanisms affecting ACTH secretion in patients with *PROP1* gene mutations may exist (e.g., lack of cellular cross-talk or cell damage after pituitary cell hyperplasia).

The prevalence of *PROP1* gene mutations in patients with congenital CPHD seems to be influenced by ethnic differences. Most reports indicate that in the Caucasian population *PROP1* gene mutations are frequently found in patients with non-acquired, familial forms of CPHD *(19,20, 23)*. In a study reported from the Czech Republic, 18 of 74 patients with CPHD had homozygous or heterozygous mutations of the *PROP1* gene *(12)*. However, Rainbow et al. *(11)* from England found 4 patients with Pit1 mutations but *PROP1* gene abnormality was absent in a cohort study of 27 children with CPHD. In a study of McLennan et al. *(9)* two *Pit1* mutations, but no *PROP1* mutation were detected in 33 Australian CPHD patients. In contrast to these reports, our study indicates a high prevalence of *PROP1* gene mutations in Hungarian CHPD patients who had GH deficiency and at least one other pituitary hormone defect. The difference between other studies and ours may be related to the great variability of the clinical phenotype of CHPD patients, or it may be the consequence of ancestral *PROP1* gene mutations that spread in a geographical or ethnic-specific manner. In this respect it seems important that all *PROP1* gene mutations detected in our patients have been previously described and that the most frequent mutations in our patients were also frequently reported in previous studies.

Although GH deficiency has been considered as the most common finding in patients with CHPD, Reynaud et al. *(24)* reported three brothers with homozygous W194X mutation of the *PROP1* gene, who developed an isolated hypogonadotropic hypogonadism. Another rare presenting symptom, a prolonged neonatal jaundice caused by central hypothyroidism, was also published in a patient with CPHD due to a compound heterozygous 296delGA/Q83X mutation of the *PROP1* gene *(25)*. In our study growth retardation was the primary complaint, which was severe in the majority of our patients with CPHD. When growth retardation was analyzed in our patients with and without *PROP1* gene mutations, the results indicated a significantly younger chronological age at the time of the diagnosis of GH deficiency in patients with *PROP1* gene mutations. However, the age at diagnosis and the prevalence of other pituitary hormone deficiencies were similar in patients with and without *PROP1* gene mutations.

In a recent study Simon et al. *(26)* examined the clinical and pituitary MRI findings of 60 patients with GH deficiency, including 30 patients with isolated growth hormone deficiency and 30 patients with CPHD. They found a strong association between an ectopic posterior pituitary and extrapituitary organ birth defects, but the potential significance of pituitary transcription factor mutations has not been investigated. Our study, together with some earlier observations indicate that *PROP1* gene mutations are not involved in the pathomechanism of ectopic posterior pituitary *(27,28)*.

According to an earlier report, a patient with CPHD caused by *PROP1* mutations may have an enlargement of the anterior lobe of the pituitary gland at the age of 8.8 yr that is normalized by the age of 15 yr *(29)*. In our study a transient hyperplasia of the anterior lobe of the pituitary was observed by pituitary MRI in one patient, whereas other patients had normal pituitary or pituitary gland hypoplasia. Voutetakis et al. *(30)* proposed that pituitary enlargement originates from the intermediate lobe. In animal models Ward and coworkers *(31)* suggested a progenitor migration defect in the pituitary gland.

In summary, our observations firmly indicate that *PROP1* gene mutations can be frequently detected in Hungarian patients with CPHD, who have GH deficiency and at least one other pituitary hormone defect. The reason for the discrepancy between our results and some previous reports showing a low prevalence of this inherited gene defect remains to be investigated.

Materials and Methods

A multicenter study including seven endocrinological centers was performed to screen mutations on the *PROP1* gene. Patients were selected over a period of 3 yr and according to predefined selection criteria. Patients were selected on the basis of clinical and hormonal evidence of childhood-onset GH deficiency combined with at least one other pituitary hormone defect in the absence of an identified cause of hypopituitarism. Patients were classified as familial or sporadic cases according to family history. There was no data of patients born to consanquineous parents. A total of 35 CPHD patients (girls/women, 13; boys/men, 22) from 32 unrelated families, including 3 families with 2 affected siblings in each family, were studied. None of the studied patients had a history of systemic chronic illness, chromosomal abnormality, brain tumor, central nervous system surgery, or cranial–neck irradiation. Written medical records were used to collect data of perinatal history and parental height. Bone age was estimated using Greulich–Pyle standards. Mean height standard deviation score for chronological age was calculated on the basis of national standards

(32). The mean chronological age at the time of diagnosis of GH deficiency was 7.7 ± 3.6 yr. Genetic testing was performed at the age of 21.8 ± 9.3 yr.

Assessment of anterior pituitary hormone production was based on serum basal hormone levels and on the results of standard provocation tests. All hormone assays were performed using commercial radioimmunoassay (RIA) kits. In all patients two standard pharmacologic provocative tests, which always included an insulin tolerance test, were used at the time of the diagnosis of childhood-onset GH deficiency to determine GH secretory capacity (insulin tolerance test plus arginine test in 25 patients, insulin tolerance test plus levodopa test in 9 patients, and insulin tolerance test plus clonidine test in 1 patient). Childhood GH deficiency was defined as peak serum GH levels lower than 7 ng/mL. The majority of patients had severe GH deficiency, as 32 of the 35 patients had peak growth hormone levels lower than 2 ng/mL. All patients older than 18 yr of age at the time of the genetic study ($n = 22$) were retested for GH secretory capacity and the results in all patients confirmed a severe GH deficiency with a peak serum GH level lower than 3 ng/mL). TSH deficiency was diagnosed on the basis of low serum T4 or fT4 level combined with disproportionally low serum TSH level. In most cases a TRH test combined with serum TSH and prolactin measurements was also performed. From the beginning of the peripubertal age clinical signs of puberty were documented and correlated with serum gonadotropin (FSH, LH) and sex steroid (testosterone and estradiol) levels. In most cases standard GnRH provocative testing was also performed.

Serum cortisol levels were studied in the morning between 0800 and 0900 and during insulin-induced hypoglycemia. Adrenal deficiency was defined when serum cortisol concentration was below 550 nmol/L in response to insulin-induced hypoglycemia. Plasma ACTH levels were also determined when signs of adrenal insufficiency were noted. Pituitary morphology based on radiological findings was analyzed retrospectively.

Pituitary magnetic resonance imaging (MRI) with T1- or T2-weighting was performed in 22 patients, brain computer tomography (CT) in 7 patients, and sella X-ray in 3 patients. Written informed consent for genetic testing was obtained from the patients or from the parents. Genomic DNA was extracted from peripheral blood leukocytes using the DNA isolation kit for mammalian blood (Boehringer Mannheim Corporation, Indianapolis, IN, USA). Mutation analysis of the *PROP1* gene was carried out using polymerase chain reaction (PCR) followed by direct bidirectional sequencing of the PCR-amplified DNA. The oligonucleotide primer pairs used for PCR amplification of exons 1–3 of the *PROP1* gene were as described by Deladoey et al. *(19).* The PCR-amplified DNA was purified using a commercial kit (High pure PCR product, Roche Diagnostics, Mannheim, Germany).

For direct sequencing of exons of the *PROP1* gene, an automated LiCOR IR2 sequencer (Li-COR, Inc., Lincoln, NE, USA), Cycle-sequencing kit with 7-deaza (Pharmacia Biotech, Uppsala, Sweden), and standard IRD-labeled sequencing primers, or the ABI Prism DNA Sequencer (Perkin Elmer Applied Biosystems, Boston, MA, USA) and the BigDye Terminator Cycle Sequencing kit were used. Steps for both sequencing methods were carried out according to the manufacturers' instructions.

Data are presented as means ± SD. All data are presented for 35 patients unless indicated otherwise. Comparisons of clinical and hormonal data were performed using independent-samples *t*-test of SPSS software package (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

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