

Frequency of mutations in PROP-1 gene in Turkish children with combined pituitary hormone deficiency

Nurgün Kandemir¹, Doğuř Vurallı¹, Ekim Tařkırın², Nazlı Gönç¹, Alev Özön¹, Ayfer Alikayıfođlu¹, Engin Yılmaz²

¹Pediatric Endocrinology Unit, Department of Pediatrics, and ²Department of Medical Biology, Hacettepe University Faculty of Medicine, Ankara, Turkey. E-mail: dogus.vuralli@hacettepe.edu.tr

SUMMARY: Kandemir N, Vurallı D, Tařkırın E, Gönç N, Özön A, Alikayıfođlu A, Yılmaz E. Frequency of mutations in PROP-1 gene in Turkish children with combined pituitary hormone deficiency. Turk J Pediatr 2012; 54: 570-575.

Mutations in the prophet of Pit-1 (PROP-1) gene are responsible for most of the cases of combined pituitary hormone deficiencies (CPHD). We performed this study to determine the prevalence of PROP-1 mutations in a group of Turkish children with CPHD. Fifty-three children with the diagnosis of CPHD were included in this study. Clinical data were obtained from medical files, and hormonal evaluation and genetic screening for PROP-1 mutations were performed. A homozygous S109X mutation was found in the second exon in two brothers, and they had growth hormone (GH) and thyroid-stimulating hormone (TSH) deficiencies and normal prolactin levels. In the third exon of the PROP-1 gene, a heterozygous A142T polymorphism was found in 14 patients and a homozygous A142T polymorphism was found in 3 patients. In the first exon, a homozygous A9A polymorphism was found in 7 patients and a heterozygous A9A polymorphism was found in 31 patients. We assumed that mutations in the PROP-1 gene in cases with CPHD were expected to be more prevalent in our population due to consanguinity, but it was found that these mutations were far less than expected and that it was rare in non-familial cases.

Key words: hyposecretion syndrome, anterior pituitary, prophet of Pit-1, paired-like homeodomain transcription factor, human, growth hormone, pituitary.

Several transcription factors, signalling molecules and regulators take roles in the development of the pituitary gland. POU1F1 (Pit-1), prophet of Pit-1 (PROP-1), HESX-1, LHX3, LHX4, and SOX3 are the best known transcription factors encoded by homeobox genes. These genes are shown to be responsible for combined pituitary hormone deficiency (CPHD). Although the prevalence of CPHD is 1/8000 live births, mutation analysis of POU1F1, PROP-1 and HESX-1 show that the incidence of mutation in sporadic forms of CPHD is very low¹⁻³. Among all the transcription factors, PROP-1 mutations are responsible for most of the cases of CPHD⁴. PROP-1 mutations are not very common in sporadic cases, but they are reported in 30-50% of familial cases^{5,6}.

In the literature, there are large series investigating mutations in patients with CPHD. However, in our country, genotypic features are

not yet described, and there are no data showing the prevalence of the mutations in the genes encoding transcription factors. Furthermore, the frequency of PROP-1 gene mutations among Turkish children with CPHD is not known. In our country, consanguineous marriage is still common and leads to a high frequency of autosomal recessive cases. We performed this study to determine the prevalence of PROP-1 mutations in a group of Turkish patients with CPHD. Knowing the high prevalence of rare genetic diseases in Turkey due to consanguinity, we hypothesized that PROP-1 mutations would be expected to be more prevalent in our population.

Material and Methods

Fifty-three children with the diagnosis of CPHD were included into this study. All children had at least one anterior pituitary

hormone deficiency in addition to growth hormone (GH) deficiency, and were therefore diagnosed as having CPHD. The medical files of the children were examined, and medical information including the age at diagnosis, sex, birth history, parental consanguinity, history of siblings, additional medical problems, hormonal profiles of the patients, and the magnetic resonance imaging (MRI) of the pituitary gland were recorded.

Hormonal assays were done by commercial radioimmunoassay (RIA) kits. GH was measured after clonidine and L-dopa stimulation, and GH deficiency was diagnosed when peak GH level was <10 ng/ml in response to both of these provocative tests. When serum T4 level was <12 pmol/L (0.93 ng/dl), if serum thyroid-stimulating hormone (TSH) concentration was inappropriately low (TSH <4.2 μ IU/ml), it was defined as TSH deficiency. When basal plasma cortisol level measured at 08:00 a.m. was <15 μ g/dl, low-dose adrenocorticotrophic hormone (ACTH) stimulation test was performed, and when peak cortisol response in low-dose ACTH test was <19.6 μ g/dl, ACTH deficiency was diagnosed. Serum prolactin (PRL) levels were also measured (normal range: 1.9-25 ng/ml). The gonadotropin axis was evaluated only in the pubertal age group. Pituitary MR imaging was also performed.

Genomic DNA was extracted from EDTA anticoagulated peripheral blood according to a standard method. All three exons and the exon-intron boundaries of the PROP-1 gene were sequenced in all study groups. Sequencing reaction was performed with BigDye Terminator sequencing kit 3.1 (PE Applied Biosystems, CA, USA) according to the manufacturer's protocol. Reaction products were analyzed with Applied Biosystems 3130 Genetic Analyzer (Table I). In those patients whose children had homozygous A142T polymorphism in the

third exon of PROP-1 gene, mutation analysis was also performed.

Results

Clinical Characteristics

Fifty-three patients with CPHD were taken into this study. The ages of the patients at the diagnosis were between 1.5 months and 15.9 years, with a mean age of 7.87 ± 5.15 years. Thirty-six subjects were males, and 17 were females. There were two brothers from the same family in the study. Consanguinity was present in 40% of the patients (21/52 families).

It was learned that seven patients were born premature, while the others were born at term. Eight patients were born via cesarean section, while the others were born via spontaneous vaginal delivery. Three patients had a history of asphyxia and difficult birth. Three patients had hypoglycemia in the newborn period, 4 had mental and motor retardation, 3 had epilepsy, 1 had hearing loss, and 1 had hypospadias.

All patients had TSH deficiency in addition to the GH deficiency when they were evaluated for the other pituitary hormone deficiencies. Twenty-two patients had ACTH deficiency. Gonadotropin deficiency was investigated only in the pubertal age group, and 12 had gonadotropin deficiency. Three patients had extremely low PRL levels. Hormonal evaluation of the patients is shown in Figure 1.

Magnetic resonance imaging (MRI) of the pituitary area was performed in 45 patients to investigate the etiology of the GH deficiency. The MRI findings are given in Table II.

Analysis of the Mutations in PROP-1 Gene

No mutation was found in the first exon of the PROP-1 gene in any of the patients. In 2 patients only, a homozygous S109X mutation was found in the second exon. The sequence

Table I. Amplification Primers and Annealing Temperature

	Primers	Amplicon	Annealing
Exon 1	5'ACA CAC ACA TTC AGA GAC AGA G3' 5'TTC TCC TCC TAA CCT TCT TC 3'	340 bp	55°C
Exon 2	5' TGG GAG ACA GAG CAA GAC 3' 5' TCT ATG ATA GCA CCA AAG AAA 3'	339 bp	55°C
Exon 3	5' TCT TGT CAT TGG AGT AGG G 3' 5' CAT AGA TGG AAA GGG AGC C 3'	597 bp	55°C

Table II. Magnetic Resonance Imaging of the Patients

MRI findings	Number of patients
Pituitary hypoplasia, agenesis of the infundibulum, posterior pituitary ectopia	30
Pituitary hypoplasia	7
Normal	7
Mass in the pituitary gland	1
Total	45

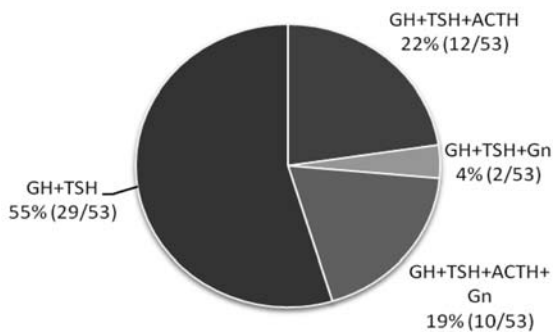


Figure 1. Hormone deficiencies in probands screened for PROP-1 mutations.

*Serum prolactin level was very low (<0.6 ng/ml) in 10% (3/29) of the patients with combined GH and TSH deficiencies.

*Abbreviations: GH (Growth hormone), TSH (Thyroid-stimulating hormone), ACTH (Adrenocorticotropic hormone), Gn (Gonadotropin)

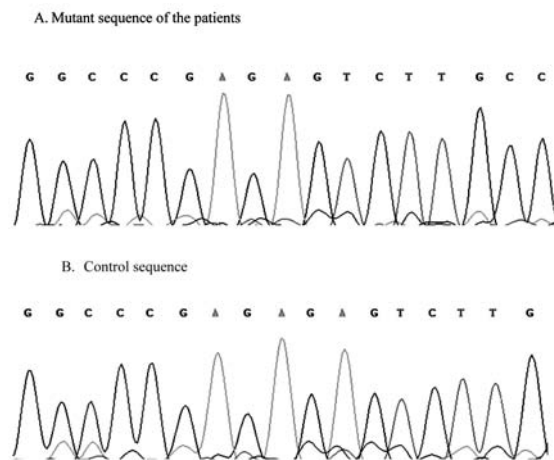


Figure 2. The sequence analysis showing S109X mutations.

analysis of these patients is given in Figure 2. These two patients were brothers, and they had GH and TSH deficiency and had normal PRL levels. Since they were in the prepubertal stage, they could not be evaluated for gonadotropin deficiencies. Both of these patients had similar phenotypic features and there was consanguinity in the family.

In the third exon of the PROP-1 gene, a heterozygous A142T polymorphism was found in 14 patients and a homozygous A142T polymorphism was found in 3 patients. When evaluating the parents of these three patients for this polymorphism, one of the parents was homozygote in two of these families and both of the parents were heterozygote in one family.

In the first exon, 7 patients were homozygote and 31 patients were heterozygote for the A9A polymorphism.

Discussion

Prophet of Pit-1 (PROP-1) is a transcription factor expressed in the Rathke pouch and is essential for the expression of another transcription factor called POU1F1 (Pit-1). PROP-1 activates POU1F1, which is the human homolog of Mouse Pit-1. PROP-1 deficiency results in deterioration in the function of somatotropes, lactotropes, thyrotropes and gonadotropes, and in the follow-up corticotropes. The PROP-1 gene is made up of three encoding exons and was first described by Sornson et al.⁷ in 1996. This gene is located on chromosome 5q in humans and codes a protein made up of 226 amino acids.

In 1998, Wu et al.⁸ described four families presenting with CPHD and having mutations in the PROP-1 gene. Thus, it was shown for the first time that PROP-1 mutations cause CPHD. PROP-1 gene mutations are the most

commonly seen mutations in the patients with CPHD. It is reported that the clinical features and hormonal deficiencies may vary between cases. In PROP-1 mutations, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and possible late-onset ACTH deficiencies are seen in addition to GH, PRL and TSH deficiencies, which are also observed in POU1F1 mutations. GH and TSH deficiencies are the first findings, and they are usually observed after one year of age. GH deficiency usually precedes TSH deficiency. Gonadotropin deficiency may be seen after puberty and causes pubertal arrest. ACTH deficiency is the most recently described deficiency and is only observed in some of the cases⁹. Since ACTH deficiency occurs as a late component, it is recommended to follow these cases for this deficiency. Individuals with POU1F1 mutations present with earlier and more severe deficiencies, whereas individuals with PROP-1 mutations present with late-onset and less severe deficiencies. In the MRI of the cases with PROP-1 mutation, the pituitary gland is usually normal or hypoplastic. However, thickness of the pituitary gland may be increased sometimes and an appearance of pituitary mass may be seen^{10,11}.

PROP-1 gene mutations are the most common mutations in recessively inherited CPHD in the cases with European origin. In contrast, it is rarely reported in cases with Asian origin. In this study, PROP-1 mutations in the patients with CPHD were investigated, and among 53 patients in 52 families, a previously described homozygous S109X mutation was determined in two brothers. In the other studies performed, the prevalence of PROP-1 mutations was reported to be 1.1% in sporadic cases and as 29.5% in familial cases⁵. In our study, this mutation was found in only two of a total of 53 cases, which was far less than the frequency reported in the literature.

In the study performed by Reynaud et al.⁶, no mutation in the PROP-1 gene was found in 111 patients investigated who had pituitary stalk interruption syndrome. According to the literature, if extrapituitary malformations such as pituitary stalk interruption syndrome exist on MRI, there is no possibility of mutations in PROP-1, POU1F1, or LHX3 genes. In the cases with posterior pituitary ectopia, the posterior pituitary lobe could be any place

in the normal pathway of the infundibulum. Posterior pituitary ectopia usually accompanies a hypoplastic anterior pituitary gland. The function of the posterior pituitary gland is nearly normal and diabetes insipidus is rarely seen in these cases. No mutation in the PROP-1 gene was found in the ectopic posterior pituitary gland cases in the literature². In all the CPHD cases defined in the literature who had PROP-1 mutations, a normal pituitary stalk and normally located posterior lobe were present on MRI^{11,12}. In our study, we could not detect any mutation in the PROP-1 gene in the cases with ectopic posterior pituitary, agenesis of the infundibulum and anterior pituitary hypoplasia in the MRI. This finding is similar to that reported in the literature.

In the study done in the West Midlands, UK, Rainbow et al.¹ evaluated 27 children from 26 unrelated families and did not detect any mutations in PROP-1, but identified two mutations in the POU1F1 gene in three familial cases. In this study, we found that PROP-1 is a rare cause of CPHD in our population. Maybe in our population, mutations in the genes other than PROP-1, such as mutations in the POU1F1 gene, can be higher in frequency, as was found in the study by Rainbow et al.¹

In the study from Australia by McLennan et al.¹³, no PROP-1 mutations were reported in the 33 children investigated. Only two of the cases were related in that study, similar to ours. No PROP-1 mutation was detected in that study, whereas in our study, mutations were detected in the two cases who were related. In another study by Deladoey et al.¹⁴, PROP-1 mutations were found in 35 of 73 cases (48%). This is the highest ratio reported in the literature. In their study, 35 cases with PROP-1 mutation were from 18 families. There were many familial cases, which may explain why they found PROP-1 mutation to be more common. When taking into account only non-familial cases, PROP-1 mutations were found in 32% of non-related affected patients in the same study. Cogan et al.¹⁵ found homozygous 301-301 del AG mutation in 5 of 10 familial cases (50%), and in only 2 of 21 sporadic cases (9.5%). Likewise, Vieira et al.¹⁶ found PROP-1 mutations were frequent in familial forms of CPHD, and they concluded that patients who are born from consanguineous parents

should be investigated for PROP-1 mutations. In our study, there was only one family with two brothers affected, and we showed PROP-1 mutation in those two brothers. Our study supported the fact that in familial cases, the presence of PROP-1 mutations is likely.

We found increased incidence of PROP-1 polymorphism in our population. Fourteen patients with heterozygous and 3 patients with homozygous A142T polymorphism in the third exon and 7 patients with homozygous and 31 patients with heterozygous A9A polymorphism in the first exon were detected. A similar increased incidence of PROP-1 polymorphism has been defined in the literature. In the cohort by Rainbow et al.¹, common polymorphisms were also identified, but no mutation in the PROP-1 gene was detected, as was the case with our study.

In most of the studies performed to date, a hot spot for CPHD was defined. Three tandem repeats of the dinucleotides GA at the location 296-302 in the PROP-1 gene was the hot spot described, and many studies from different countries confirmed this hot spot^{16,17}. Analysis of the PROP-1 gene in 14 Russian cases with CPHD, whose Pit-1 gene analysis was found to be normal, showed a two base-pair (bp) homozygous deletion (296del GA) in the second exon of three cases within two families¹⁸. This deletion led to a stop codon instead of serine and resulted in a truncated PROP-1 protein. The same investigators¹⁹ also reported another 2 bp deletion (149delGA) that was present in the compound heterozygous state with 296 del GA. This mutation also led to formation of a premature stop codon, by means of which, the loss of the function was complete. Deladoey et al.¹⁴ also described these two frameshift mutations leading to S109X amino acid change in the second exon of the PROP-1 gene. We also found this same S109X mutation in two siblings.

In the CPHD cases with genetic origin, frequency of consanguineous marriage is high, and it is highly possible for these cases to be familial and to have intact pituitary stalk and normally located posterior lobe.

In conclusion, mutations in the PROP-1 gene in cases with CPHD in our country are thought to be far less common than described in the cases with European origin. In this study,

which shows the frequency in our country, a homozygous mutation was shown in only one family, which leads us to think that the frequency is less than expected. Mutations in different genes that have a role in the development and function of the pituitary gland, which we did not investigate, are probably responsible for these cases in our country; therefore, new studies must be performed to evaluate these mutations.

REFERENCES

1. Rainbow LA, Reest SA, Shaikh MG, et al. 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* 2005; 62: 163-168.
2. Kim SS, Kim Y, Shin YL, Kim GH, Kim TU, Yoo HW. Clinical characteristics and molecular analysis of PIT1, PROP1, LHX3, and HESX1 in combined pituitary hormone deficiency patients with abnormal pituitary MR imaging. *Horm Res* 2003; 60: 277-283.
3. Cruz JB, Nunes VS, Clara SA, Perone D, Kopp P, Nogueira CR. Molecular analysis of the PROP1 and HESX1 genes in patients with septo-optic dysplasia and/or pituitary hormone deficiency. *Arq Bras Endocrinol Metabol* 2010; 54: 482-487.
4. Kelberman D, Turton JP, Woods KS, et al. Molecular analysis of novel PROP1 mutations associated with combined pituitary hormone deficiency (CPHD). *Clin Endocrinol (Oxf)* 2009; 70: 96-103.
5. Turton JP, Mehta A, Raza J, et al. Mutations within the transcription factor PROP-1 are rare in a cohort of patients with sporadic combined pituitary hormone deficiency. *Clin Endocrinol (Oxf)* 2005; 63: 10-18.
6. Reynaud R, Gueydan M, Saveanu A, et al. Genetic screening of combined pituitary hormone deficiency: experience in 195 patients. *J Clin Endocrinol Metab* 2006; 91: 3329-3336.
7. Sornson MW, Wu W, Dasen JS, et al. Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. *Nature* 1996; 384: 327-333.
8. Wu W, Cogan JD, Pfaffle RW, et al. Mutations in PROP1 cause familial pituitary combined hormone deficiency. *Nat Genet* 1998; 18: 147-149.
9. Agarwal G, Bhatia V, Cook S, Thomas PQ. Adrenocorticotropin deficiency in combined pituitary hormone deficiency patients homozygous for a novel PROP1 deletion. *J Clin Endocrinol Metab* 2000; 85: 4556-4561.
10. Voutetakis A, Argyropoulou M, Sertedaki A, et al. Pituitary magnetic resonance imaging in 15 patients with Prop1 gene mutations: pituitary enlargement may originate from the intermediate lobe. *J Clin Endocrinol Metab* 2004; 89: 2200-2206.
11. Crone J, Pfaffle R, Stobbe H, Prayer D, Gomez I, Frisch H. Familial combined pituitary hormone deficiency caused by PROP-1 gene mutation. Growth patterns

- and MRI studies in untreated subjects. *Horm Res* 2002; 57: 120-126.
12. Mody S, Brown MR, Parks JS. The spectrum of hypopituitarism caused by PROP1 mutations. *Best Pract Res Clin Endocrinol Metab* 2002; 16: 421-431.
 13. McLennan K, Jeske Y, Cotterill A, et al. Combined pituitary hormone deficiency in Australian children: clinical and genetic correlates. *Clin Endocrinol (Oxf)* 2003; 58: 785-794.
 14. Deladoey J, Fluck C, Buyukgebiz A, et al. 'Hot Spot' in the PROP1 gene responsible for combined pituitary hormone deficiency. *J Clin Endocrinol Metab* 1999; 84: 1645-1650.
 15. Cogan JD, Wu W, Phillips JA 3rd, et al. The PROP1 2-base pair deletion is a common cause of combined pituitary hormone deficiency. *J Clin Endocrinol Metab* 1998; 83: 3346-3349.
 16. Vieira TC, Boldarine VT, Abucham J. Molecular analysis of PROP1, PIT1, HESX1, LHX3, and LHX4 shows high frequency of PROP1 mutations in patients with familial forms of combined pituitary hormone deficiency. *Arq Bras Endocrinol Metabol* 2007; 51: 1097-1103.
 17. Mendonca BB, Osorio MG, Latronico AC, Estefan V, Lo LS, Arnhold IJ. 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 Metab* 1999; 84: 942-945.
 18. Fofanova OV, Takamura N, Kinoshita E, et al. A mutational hot spot in the PROP-1 gene in Russian children with combined pituitary hormone deficiency. *Pituitary* 1998; 1: 45-49.
 19. Fofanova O, Takamura N, Kinoshita E, et al. Compound heterozygous deletion of the PROP-1 gene in children with combined pituitary hormone deficiency. *J Clin Endocrinol Metab* 1998; 83: 2601-2604.