**ORIGINAL ARTICLE**

**PROP1 gene analysis in Portuguese patients with combined pituitary hormone deficiency**

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**Summary**

**Objective** Mutations of the PROP1 gene lead to combined pituitary hormone deficiency (CPHD), which is characterized by a deficiency of GH, TSH, LH/FSH, PRL and, less frequently, ACTH. This study was undertaken to investigate the molecular defect in a cohort of patients with CPHD.

**Design, patients and measurements** A multicentric study involving 46 cases of CPHD (17 familial cases belonging to seven kindreds and 29 sporadic cases) selected on the basis of clinical and hormonal evidence of GH deficiency, central hypothyroidism and hypogonadotrophic hypogonadism, in the absence of an identified cause of hypopituitarism. Mutations of PROP1 were investigated by DNA sequencing. Clinical, hormonal and neuroradiological data were collected at each centre.

**Results** PROP1 mutations were identified in all familial cases: five kindreds presented a c. 301–302delAG mutation, one kindred presented a c. 358C→T (R120C) mutation and one presented a previously unreported initiation codon mutation, c. 2T→C. Of the 29 sporadic cases, only two (6·9%) presented PROP1 germline mutations (c. 301–302delAG, in both). Phenotypic variability was observed among patients with the same mutations, particularly the presence and age of onset of hypocortisolism, the levels of PRL and the results of pituitary imaging. One patient presented a sellar mass that persisted into adulthood.

**Conclusions** This is the first report of a mutation in the initiation codon of the PROP1 gene and this further expands the spectrum of known mutations responsible for CPHD. The low mutation frequency observed in sporadic cases may be due to the involvement of other unidentified acquired or genetic causes.
Subjects and methods

Subjects

A multicentric study was launched in Portugal to screen for mutations of the PROP1 gene in patients with CPHD. Endocrinologists based at national endocrine referral centres, serving the northern, central and southern regions of the country, were invited to select patients from their outpatient clinics, over a period of 2 years and according to predefined selection criteria. Patients were selected on the basis of clinical and hormonal evidence of GH deficiency, central hypothyroidism and hypogonadotrophic hypogonadism, in the absence of an identified cause of hypopituitarism, and classified according to family history, as familial or sporadic cases.

A total of 46 cases were assessed, 17 belonged to seven apparently unrelated families with a positive history of CPHD and 29 were sporadic cases. As the majority of participating centres were adult endocrine units, only eight patients were under 18 years of age at the time of this study. All selected patients agreed to be included in the study. As there was no central registry of CPHD patients, an undetermined number of patients under the care of nonendocrine specialties may have been excluded from this study.

Clinical, hormonal and neuroradiological data were collected from each patient by physicians at each referring centre. The biochemical study was performed by measuring free T3 and T4, IGF-1, oestradiol/testosterone and PRL at baseline. As patients were studied at various centres using different commercial hormonal assays, normal values for each centre were taken into account. Anterior pituitary function was assessed by determining the response of TSH to TRH (200 µg, iv), the LH and FSH responses to GnRH (100 µg, iv), the cortisol response to synthetic ACTH1–24 (250 µg, iv) and the GH, cortisol and ACTH responses to an insulin tolerance test (0·1 U/kg, iv), as described previously. Onset of ACTH deficiency was considered retrospectively to be the earliest age at which the stimulated cortisol levels fell below 550 nmol/l.
Genomic analysis of the PROP1 gene

Venous blood samples were obtained after informed consent using guidelines approved by the local research ethics committee and genomic DNA was extracted using standard methods. Each of the three exons of the PROP1 gene was amplified by polymerase chain reaction (PCR) using previously described flanking primer sequences. In brief, 100–200 ng of genomic DNA were used in 50 μl reactions containing 25 pmol of each primer, 1 U of Taq DNA polymerase, 200 μM of each dNTP, 50 mM KCl, 1½ mM MgCl₂, 10 mM Tris–HCl (pH 9·0). Cycle conditions consisted of initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, with a final extension step of 72 °C for 10 min. The PCR products were purified using the QiAquick PCR purification kit (Qiagen) and direct sequencing of both strands of the products was carried out on a CEQ8000 capillary sequencer using the CEQ Dye Terminator Cycle Sequencing Quick Start Kit (Beckman Coulter, Fullerton, CA, USA) with the same PCR primers.

Mutations were confirmed by repeating the PCR followed by the use of appropriate restriction endonucleases for the nucleotide changes and gel electrophoresis of the digested fragments. Nomenclature for nucleotide changes followed the standard format c. XXX, where c indicates the cDNA sequence and XXX represents the altered nucleotide(s) of that sequence. Previously unreported nucleotide changes were screened for in a panel of 55 normal individuals (110 nucleotide(s) of that sequence. Previously unreported nucleotide changes were screened for in a panel of 55 normal individuals (110 alleles) to assess the possibility of common polymorphisms.

Results

Germline mutations of the PROP1 gene were found in all familial cases of CPHD and in two sporadic cases (Fig. 2). A 2-bp deletion, c. 301–302delAG, was found in five families (F1, F2, F4, F6 and F9) and in both sporadic cases (F3, F8). One family (F7) had a c. 358C → T mutation and another (F5) had an initiation codon c. 2T → C mutation. All mutations were present in homozygosity. No other mutations of the coding sequence of the gene were present in the remaining 27 sporadic cases. Mutations were also confirmed by the use of restriction endonucleases. The c. 301–302delAG mutation generated a restriction site for BgL1 that resulted in fragments of 247, 133 and 34 bp, instead of the normal PCR product of 416 bp. The c. 358C → T mutation generated a PstI restriction site that resulted in fragments of 448 and 131 bp, instead of the normal PCR product of 579 bp. The c. 2T → C mutation eliminated a restriction site for NcoI that would normally cleave the 326-bp PCR product into fragments of 197 and 129 bp (Fig. 3).

All 19 patients with germline mutations (Table 2) had presented with growth retardation and central hypothyroidism during childhood and complete absence of puberty. GH, TSH and LH/FSH deficiencies were demonstrated in all of these patients. PRL levels were under 5 ng/ml, in 15 out of 19 cases, and ACTH impairment was present in eight out of 19 cases, with an age of onset ranging from 11 to 36 years (Table 2). Magnetic resonance imaging (MRI) scans demonstrated pituitary hypoplasia or empty sella in all except one patient aged 27 years, who presented a nonenhancing sellar mass with high-intensity in T1 and low signal in T2 (Fig. 4). This mass was initially detected during a first MRI performed at 16 years of age, and at that time measured 10 × 10 × 12 mm (height × length × width), with extension above the sellar fossa. This mass maintained these characteristics up to the age of 21 years (Fig. 4) and then underwent a reduction in volume until its current size of 5 × 5 × 7 mm.

Discussion

The most frequent mutation found in this study was c. 301–302delAG, which was present in five out of seven kindreds and in the only two sporadic cases with PROP1 germline mutations. This 2-bp deletion occurs in a dinucleotide repeat mutational hotspot and is the most frequently reported mutation in patients with CPHD, constituting over half of all reported PROP1 mutations in CPHD.

Fig. 2 Family trees of CPHD patients found to harbour PROP1 germline mutations. Individuals are represented as males (squares), females (circles), affected (filled symbols), unaffected (open symbols) and deceased (oblique line through symbol). The double line represents a consanguineous marriage. Patients F3 II-1 and F8 II-1 presented as sporadic cases (i.e. no family history of CPHD).

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codon 109, leading to the loss of the DNA-binding homeodomain and the C-terminal transactivation domain of PROP1. Another mutation found in this study was located in exon 3 and led to a C → T nucleotide transition at position c.358, resulting in the substitution of an arginine by a cysteine at codon 120, which has been shown to severely affect the DNA binding and transactivation ability of PROP1.

Our study revealed a novel initiation codon mutation of the PROP1 gene, which is also the first reported mutation in exon 1 of this gene. Several examples of mutations in the ATG initiation codon and in the flanking consensus sequence have been reported in association with human disease. Unlike some other organisms, eukaryotic ribosomes rarely initiate translation at a codon other than ATG. For some genes, elimination of the initiation codon can result in the use of an alternative downstream ATG codon as the translational initiation site. There are no ATG triplets upstream of the normal initiation site in the PROP1 cDNA deposited in GenBank (accession NM_006261) and there are only two that are located downstream within the coding region. The first is located at nucleotides c.518–520 but is out of frame. The second is located at nucleotides c.640–642 and is in frame, but lacks the flanking consensus sequence, and even if translation occurred, the resultant peptide would only have the terminal 13 residues instead of the normal 226 amino acids that constitute the fully translated protein. The encountered mutation therefore appears to lead to a null allele that abolishes the translation of the PROP1 protein.

All patients with these mutations had deficiency of GH, TSH and LH/FSH; however, there was phenotypic variability in terms of the levels of PRL and the presence of ACTH deficiency, even among patients with the same mutation. Low levels of PRL were found in the majority of patients. Although PRL deficiency has been reported to be variable in several studies, it has not been given as much emphasis as other hormone deficiencies. This may be due to the lack of an appropriate definition of this deficiency and to the apparent lack of clinical consequences, with perhaps the exception of puerperal alactogenesis following successful fertility treatment. In the Ames mouse, PRL was not detectable by immunoprecipitation but PRL-immunoreactive cells could be identified, so it is conceivable that in humans some residual function may occur. ACTH impairment has been increasingly recognized as a late-onset complication in
patients with *PROP1* mutations. A previous study of 10 patients with
the c. 301–302delAG mutation showed that five out of six patients
who were over 40 years of age developed ACTH deficiency compared
to only one out of four patients below the age of 40.18 Several of our
oldest patients (up to the age of 66 years) have not developed this
deficiency whereas others presented abnormal cortisol responses as
early as 11 years of age. The cause of ACTH deficiency, which does
not occur in the Ames mouse model, is currently unknown but may
indicate a possible role of *PROP1* in the differentiation and/or main-
tenance of corticotroph cells. In agreement with previous reports,17
none of our patients with the mutation c. 358C → T presented this
deficiency and this may be related to some degree of residual activity
conferred by this missense mutation.3

Pituitary size was also variable among patients. MRI demonstrated
pituitary hypoplasia or empty sellae in all patients except one, who
presented an enlarged sellar mass. Pituitary enlargement has been
frequently observed in young patients with *PROP1* mutations, but
this feature appears to be followed by degeneration and regression
of the mass, often resulting in an empty sella with a small remainder
of pituitary tissue.19 These pituitary masses, with one exception,18
have only been reported in young children and this contrasts with
MRI results that one of our patients maintained up to the age
of 21 years. In the remaining patients, because of the lack of adequate
imaging studies in childhood, it was not possible to confirm the
previous existence of any pituitary mass, although this would be
compatible with the high frequency of empty sella currently
observed in this group of patients.

Genotype–phenotype correlations were not established because of
the limited number of patients in each mutation group. However,
the phenotype associated with the novel initiation codon mutation
does not appear to differ from that conferred by the more frequent
2-bp deletion.

Only a small proportion (6-9%) of sporadic cases was found to
harbour *PROP1* mutations. The majority of cases are thus likely to
be phenocopies resulting from unrecognized acquired causes. Other
genes responsible for CPHD, such as *PIT1* and *HESX1*, were not
analysed in this study as they are normally associated with a different
phenotype, but mutations of other transcription factors such as
*LHX3* or *LHX4* or other unknown genetic defects cannot be excluded
in these patients.19

Given the mode of inheritance of autosomal recessive disorders,
most patients with mutations would not be expected to present any
family history of the disorder, as each sibling has only a 25% pro-
bability of being affected. In this study, the relatively low ratio of
mutated sporadic to familial cases suggests a possible selection bias
for familial cases at the referring centres, although this would not
alter the main conclusions of the study.

Testing for *PROP1* mutations in CPHD patients, even in sporadic
cases, may be warranted in view of the implications in their clinical
management, such as early awareness of the failure to enter spontaneous
puberty, of the risk of future ACTH deficiency and of the natural
history of any existing sellar mass.

In conclusion, in this study of a cohort of CPHD patients due to
*PROP1* mutations, we observed variable expression of PRL and ACTH
levels and an unusual case of persistence of a sellar mass into adulthood.
Furthermore, we identified the first mutation in the initiation codon
of the *PROP1* gene and this further expands the spectrum of known
mutations responsible for hypopituitarism.
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References


27 Voutetakis, A., Maniati-Christidi, M., Kanaka-Gantenbein, C.,


