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The Natural History of the R120C *PROP1* Mutation Reveals a Wide Phenotypic Variability in Two Untreated Adult Brothers with Combined Pituitary Hormone Deficiency

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Background: **Combined pituitary hormone deficiency (CPHD) corresponds to impaired production of growth hormone (GH) and other anterior pituitary hormones. The genetic form of CPHD may result from mutations in pituitary transcription factor genes, and *PROP1* is the most commonly mutated gene in these cases. Patients with *PROP1* mutations may have variable CPHD phenotypes but, because they are usually treated in childhood, the wide phenotypic variability caused by these mutations may not be thoroughly appreciated.** ***Methods:*** **Clinical follow-up and molecular analysis of *PROP1* in two adult brothers with CPHD, born from consanguineous parents, and not treated until late adulthood.** ***Results:*** **The homozygous R120C mutation was identified in the brothers. Their clinical follow-up showed a wide phenotypic variability: hypogonadism was severe and prevented pubertal development in both, but their final heights were remarkably different, pointing to different degrees in severity of GH/TSH deficiencies; cortisol deficiency developed late in both, but at least 10 yr apart.** ***Conclusions:*** **The lack of treatment in childhood and adolescence allowed the appreciation of the entire natural history of the CPHD caused by the R120C mutation, and it revealed a remarkable phenotypic variability even in siblings with a very similar genetic background.**

Key Words: Hypopituitarism; combined pituitary hormone deficiency; pituitary transcription factors; mutation, *PROP1*.

Introduction

Combined pituitary hormone deficiency (CPHD) includes a heterogeneous group of disorders characterized by impaired production of growth hormone (GH) and one or more of the other anterior pituitary hormones. Clinically, it is characterized by a combination of two or more of the following findings: short stature, hypothyroidism, impaired sexual development, and hypocortisolism. CPHD may result from acquired lesions in the hypothalamic–pituitary region (tumor, trauma, surgery, irradiation) or genetically defined conditions, or may be idiopathic. CPHD from a genetic cause has an incidence of approx 1:8000 births and is usually sporadic, but nearly 5–30% of cases are familial *(1–3)*. Mutations in the genes that encode the transcription factors (TF) involved in the organogenesis of the pituitary, *HESX1, LHX3, LHX4, PIT-1, PROP1, SOX3,* may lead to the genetic forms of CPHD *(4–10)*. In humans, *PROP-1* mutations are the most common cause of genetic CPHD, accounting for up to 50% of all cases *(3,11,12)*. PROP-1 is necessary for the activation of the *POU1F-1* gene, the human homolog of mouse *Pit-1 (13,14)*. Defects in the *PROP-1* gene may cause deficiencies in pituitary hormones produced by POU1F-1–dependent cell lineages (somatotrophs, thyrotrophs, lactotrophs), as well as gonadotrophin and corticotrophin deficiencies *(3–5,15–18)*.

The most frequent *PROP-1* mutation, which accounts for more than 50% of all *PROP-1* mutations, is a 2 bp AG deletion (301–302delAG) in a region containing three AG repeats in exon 2 (nucleotides 297–302) *(3,5,11,12,15,16, 19,20)*. This and other less frequent mutations give rise to abnormal proteins with reduced DNA binding and transactivation properties *(5,21–23)*.

In this study, we describe two Brazilian brothers with CPHD bearing the rare R120C *PROP1* mutation. Because they did not receive any hormonal replacement until late adulthood, we were able to analyze the variable phenotype of this mutation in the absence of treatment.

Results

Genomic Sequencing of the PROP-1 *Gene*

As shown in Fig. 1, direct sequencing of the PCR products showed that both affected brothers harbor a homozygous 358C>T mutation in exon 3 of the *PROP-1* gene. This mutation predicts an amino acid change at codon 120 replacing a much conserved arginine by a cysteine (R120C) in the third

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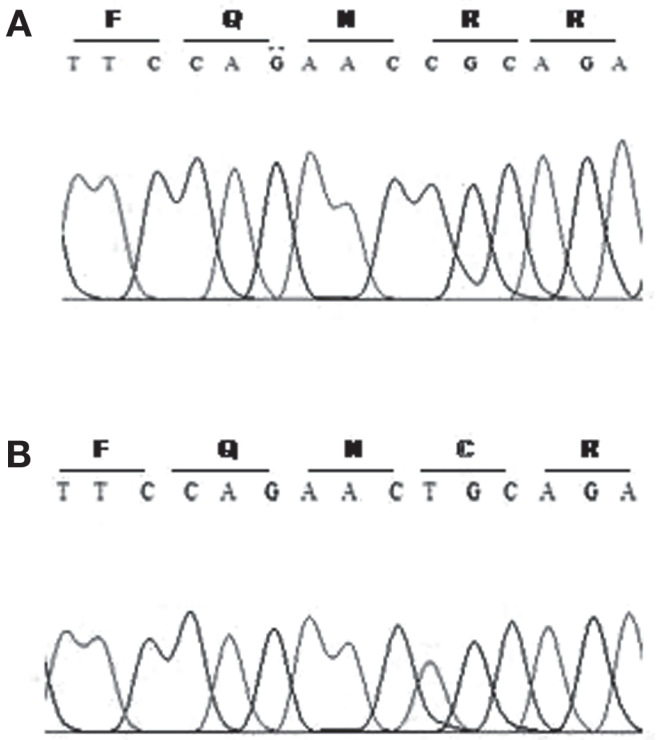

Fig. 1. Partial sequence chromatogram of exon 3 of the *PROP1* gene showing the wild type sequence (**A**) and the missense mutation 358C>T in homozygosis (**B**).

helix of the DNA-binding domain of the PROP1 protein, and the mutant protein has an eight-fold reduction in DNA binding affinity and impaired trans-activation activity *(5)*.

Discussion

The *PROP1* 358C>T (R120C) missense mutation identified in these brothers has never been described in Brazilian families and only four families, two from Switzerland, one Mexican-American, and one of Jewish–Moroccan origin, have been reported with this mutation *(5,27–29)*. The two brothers here reported developed progressive CPHD, but were only diagnosed and treated in adulthood because they grew up in an impoverished region of Brazil where medical care was very inadequate. Only in adulthood, when they moved to São Paulo, were they able to look for good medical assistance.

The late diagnosis and treatment allowed their CPHD to develop entirely and it was possible to observe the natural history of this mutation. These patients, born from first-degree cousins and, therefore, with a very close genetic background, presented a wide phenotypic CPHD variability: their gonadotropin deficiency was severe and prevented pubertal development in both; on the other hand, their final heights were remarkably different (−1.0 SD in the older brother and −3.4 SD in the younger one), pointing to different degrees in severity and/or progression of GH deficiency.

Central hypothyroidism was diagnosed at different ages, at 24 yr in the older and at 12 yr in the younger brother. It is possible that in the older brother, not only a milder GH deficiency, but also a normal thyroid function during childhood, allowed him to grow better than his younger brother in the absence of gonadal steroids. On the other hand, although cortisol deficiency only developed late in life in both, it was more precocious in the older brother who was diagnosed with cortisol deficiency at the age of 29 yr whereas the younger one developed cortisol deficiency only around 39 yr of age (Table 1). In the older brother, it is possible that the long-standing hypothyroidism and GH deficiency contributed to the absence of symptoms of secondary adrenal insufficiency, even in the presence of severely low cortisol levels (0.9 μg/dL, Table 1).

Two Swiss families with five affected members with the same *PROP1* R120C mutation described by Fluck also presented heterogeneous CPHD phenotypes *(29)*. The ages at diagnosis of hypopituitarism ranged from 9 mo to 8 yr and the pituitary hormone deficiencies developed progressively, over a variable period, following a different pattern in each individual. In one patient, the first symptom was TSH deficiency and in the others, the first abnormality was growth failure due to GH deficiency. Differently from our patients, all of them entered puberty spontaneously, showing partial gonadotropin deficiency. They received GH and levothyroxin replacement in childhood and/or adolescence and this definitely influenced their final heights *(27)*. There is another patient reported in the literature with the same *PROP1* mutation that had GH, TSH, PRL, and gonadotropin deficiencies. She received steroid replacement (birth control pills) in her mid twenties and, differently from our patients, her bone X-ray revealed complete epiphyseal closure on first evaluation. She reached a stature close to her target height (157 cm, TH = 161 cm) *(28)*. The R120C *PROP1* mutation was also identified in a third consanguineous family of Jewish–Moroccan origin. In this family, eight subjects presented GH and TSH deficiencies diagnosed at different ages (5.5–10.8 yr) and all exhibited complete gonadotropin deficiency with failure of spontaneous sexual maturation. ACTH deficiency developed only in two sisters in the 3rd and 4th decades of life. The CPHD in this family was also characterized by clinical phenotypic differences in terms of severity and time of development of hormonal deficiencies *(29)*.

The phenotypic CPHD variability seen in the two brothers of this study and in the members of the other families with this same *PROP1* mutation is intriguing, but it has been observed in many other Mendelian disorders. Just to mention one example in the area of endocrinology, patients with Kallmann syndrome belonging to a single family where one specific *FGFR1* mutation has been identified, may present a variable spectrum of phenotypes including reversible Kallmann syndrome, delayed puberty, and isolated anosmia *(30)*. In nonendocrine diseases as cystic fibro-

Table 1
Hormonal Profile of the Two Brothers with the *PROP1* R120C Mutation

Age and Height at hormone testing	FT4 (ng/dl) TSH (mU/L)	PRL ng/ml	IGF-I ng/ml	Peak GH (ng/ml) after ITT	Cortisol (ug/dl) after ITT	LH/FSH (U/L) after GnRH	Testo (ng/dl)
Older brother 29yr 170 cm (-1.0 SD)	0.6/ 4.7	<3	-	1.4	B:<0.9 P:<0.9	B:5/1 P:8/1	22
Younger brother 37yr 154 cm (-3.4 SD)	0.4/ 3.7	<3	21	-	B:7.7 (37 yr) B:2.0(39 yr)	B:<2/<1	8
Normal values	FT_4: 0.8- 2.7 TSH: 0.3-4.0	3 - 15	25-39 yr: 114-400	P: >5	B:5-25 P:>18	LH B:<14 LH P:>4-6x	300-950

sis, for example, the clinical variability of patients bearing the same mutation in the *CFTR* gene is well known, and molecular studies have shown that the presence of subtle variations in the number of nucleotide repeats in introns of the *CFTR* gene modify the action of regulatory nuclear proteins involved in *CFTR* expression *(31,32)*. Other examples of the phenotypic complexity of the monogenic disorders are some monozygotic twins, born embedded in identical genomes, who inherit an identical mutation of a specific gene. Surprisingly, in this situation, they may manifest discordant phenotypes of the disease, pointing to the presence of multiple epigenetic and/or environmental factors or processes leading to variable phenotypic manifestations *(33,34)*.

In summary, the CPHD phenotype due to the R120C *PROP1* mutation may be widely variable no matter whether the affected patients have a very close genetic background such as siblings born from first-degree cousins. The phenotypic variability of our patients could be entirely appreciated, because there was no interference of medical treatment until late adulthood. The understanding of how the same *PROP1* genotype can originate different CPHD phenotypes will improve, as the molecular research on monogenic Mendelian diseases progresses.

Patients and Methods

After obtaining approval for the study protocol from the Institutional Ethics Committee, written informed consent was obtained from the parents.

Clinical and Hormonal Evaluation

The two brothers, presently 41 and 39 yr old, were born from a consanguineous marriage (parents were first-degree cousins) and progressively developed CPHD, but were only diagnosed and treated in adulthood.

The older brother was first evaluated in our service 12 yr ago, at the age of 29 yr, complaining of lack of puberty. His past medical history was positive for learning difficulties and hypothyroidism diagnosed at the age of 24 yr, but never treated. On physical examination his height was 170 cm, between the 10th and 25th percentiles (−1.0 SD) *(24)*. His target height was not available, because his father was deceased and the patient did not know his parents' height. His pubertal Tanner stage was P_1G_1 *(25)* and he had cold skin. His bone age was 16 yr (CA = 29 yr) *(26)*. He was submitted to ITT and GnRH stimulation tests that revealed GH, ACTH, and gonadotropin deficiencies. Interestingly, the basal and peak concentrations of cortisol were very low (0.9 µg/dL, Table 1), but he had no complaints of hypocortisolism. Cortisol measurement was then repeated and the first result was confirmed. Basal levels of free T4, TSH, and PRL were compatible with central hypothyroidism and PRL deficiency (Table 1). His sellar MRI revealed a partially empty sella and a small anterior pituitary (Fig. 2).

His younger brother came to medical attention at 37 yr of age, brought by his older brother. He also had total lack of pubertal development and a non-treated hypothyroidism since the age of 12 yr. On physical exam he was P_1G_1 and his height was 154 cm (below the 3rd percentile or < −3.0 SD) *(24)*. Although he was much smaller than his older brother, his bone age was also 16 yr. Anterior pituitary hormone evaluation revealed GH and PRL deficiencies and confirmed the central hypothyroidism and gonadotropin deficiency (Table 1). He stated that he was doing well and his basal levels of cortisol were in the normal range. He was started on testosterone and levothyroxin replacement and did well. Two years later, at the age of 39 yr, he developed symptoms of hypocortisolism and hormonal tests confirmed cortisol deficiency (Table 1). Like his brother, he also had a partially empty sella and small anterior pituitary on MRI.

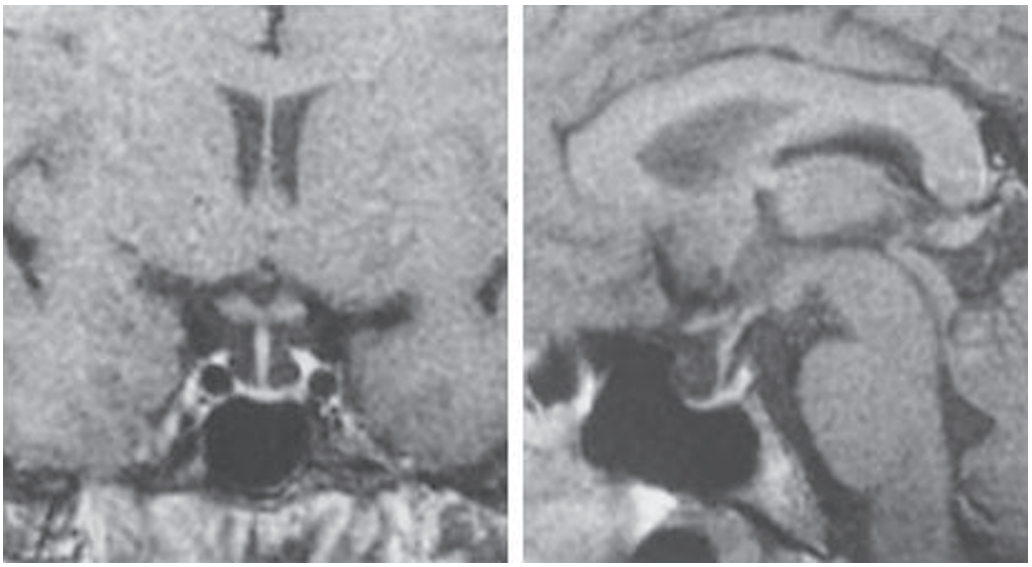

Fig. 2. Pituitary MRI of the affected older brother. Coronal and lateral T-1 weighted views show an empty sella, a normal stalk and posterior pituitary gland.

Genomic Analysis

DNA was extracted from peripheral lymphocytes of the brothers using a Quiagen Midi Kit (Quiagen), following the manufacturer's protocol. Their parents were not submitted to DNA analysis, because the father was deceased and the mother, living far, was not available.

All three exon *PROP1* genes were amplified by polymerase chain reaction (PCR). One hundred nanograms of human genomic DNA were used as template in a 100 µL PCR mixture containing 20 m*M* Tris-HCl (pH 8.4), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* deoxy-NTPs, 2.5 U *Taq* polymerase (PCR Reagent System, Life-Technologies), and 0.1 n*M* of upstream and downstream specific primers. The sequence of the PCR primers and the PCR thermal cycling program have been described elsewhere *(16)*. PCR products were analyzed in 1.8% agarose gel and purified using a PCR Product Purification Kit (Life Technologies, USA). Direct sequencing of the PCR products was carried out in both directions, using the ABI Prism Big Dye terminator cycle sequencing ready reaction version 3.0 (Applied Biosystems) in an ABI Prism 3100 DNA Sequencer (Perkin Elmer Corporation).

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