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PROP1 Mutations Cause Progressive Deterioration of Anterior Pituitary Function including Adrenal Insufficiency: A Longitudinal Analysis

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Mutations in the *PROP1* gene are the most frequent genetic defects in patients with combined pituitary hormone insufficiency. However, controversy exists about the timing and extent of pituitary insufficiency, and it remains unclear whether adrenal failure is a typical feature of this condition.

We performed a retrospective longitudinal analysis of nine patients with *PROP1* mutations who were under medical supervision at our clinic for 15.7 ± 3.4 yr. All patients initially presented with growth failure (height SD score, –3.7 ± 0.3) at a mean age of 4.9 ± 0.8 yr. They were first diagnosed with GH and TSH deficiency, and replacement therapy was instituted at 6.1 ± 1.1 and 6.8 ± 1.2 yr, respectively. All seven patients who reached pubertal age required sex hormone substitution at 15.0 ± 0.7 yr.

Repeated functional testing of the anterior pituitary axes revealed a progressive decline with age in peak levels of GH, TSH, prolactin, and LH/FSH. All patients developed at least partial adrenal insufficiency, with a gradual decline of the function of the pituitary adrenal axis and eventually required substitution with hydrocortisone at a mean age of 18.4 ± 3.5 yr.

It is concluded that anterior pituitary function in patients with *PROP1* mutations deteriorates progressively and includes adrenal insufficiency as a feature of this condition, which has important clinical relevance in childhood and adolescence. (*J Clin Endocrinol Metab* 89: 5256–5265, 2004)

COMBINED PITUITARY HORMONE deficiency (CPHD) refers to the impaired production of several anterior pituitary hormones, commonly including GH. In many pediatric patients, mutations in pituitary-specific transcription factors can be identified as the cause of the disorder (1).

Pituitary function strongly depends on an organized embryonic development with respect to the morphology of the gland, the cell populations of the five distinct types (somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, and corticotrophs), and their tightly regulated endocrine function (2). This highly complex process crucially depends on the sequential expression of a cascade of transcription factors during pituitary organogenesis. Each of these transcription factors directs the development, selective determination, differentiation, and proliferation of common precursor cell lines toward each of the five endocrine cell types within the anterior pituitary (2, 3). Hence, abrogation of a single transcription factor may result in deleterious consequences for pituitary development and endocrine function. Many mutations in transcription factors have been associated with specific combinations of missing pituitary hormones (4). More than 50% of familial CPHD of pituitary origin is attributable to mutations in one of the transcription factors involved in pituitary development, such as *PROP1*, *POU1F1*, *HESX1*, *LHX3*, and *LHX4* (5). Among the phenotypes with genetically identifiable defects, those caused by mutations in the *PROP1* gene are the most frequent, accounting for approximately 50% of genetically determined CPHD (6).

PROP1 (prophet of Pit1) expression appears early in embryonic development and is crucial for the differentiation and function of somatotrophs, thyrotrophs, gonadotrophs, and lactotrophs (7, 8). The Ames dwarf mouse is a natural animal model with a missense mutation in the *Prop1* gene that leads to attenuated DNA binding and transactivation capacity (9). Consequently, these mice are phenotypically characterized by failure of the somatotroph, thyrotroph, and lactotroph axes and to a more variable degree by failure of the gonadotroph but not the corticotroph axes (9). In humans, several independent mutations in the *PROP1* gene have been identified as a cause for CPHD (10, 11). From animal studies and the understanding of the role of *PROP1* in pituitary embryonic development and biology one may assume that human patients with *PROP1* defects also suffer from failure of the somatotroph, lactotroph, and thyrotroph axes. Although most patients reported to date show these expected endocrine deficiencies, the degree of gonadotroph failure is variable in adolescents, even though most adult patients suffer from hypogonadism (7, 8). There is even more controversy about the function of the pituitary adrenal axis in patients with *PROP1* defects. Failure of corticotroph function is not expected from *in vitro* and animal models.

Abbreviations: BMI, Body mass index; CPHD, combined pituitary hormone deficiency; GHD, GH deficiency; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; SDS, SD score.

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The objective of this study was, therefore, to analyze the prevalence of adrenal insufficiency in patients with *PROP1* defects and to characterize the temporal pattern of anterior pituitary failure. To this end we conducted a retrospective, longitudinal analysis of the clinical course of nine patients, who were continuously followed for an average of more than 15 yr at our institution and who were recently identified with *PROP1* mutations.

Patients and Methods

Patients: clinical evaluation

Patients were referred to the Auxological-Endocrine Department of the University Hospital for Children and Adolescents, Leipzig, for evaluation and treatment of growth retardation. The screening cohort of 46 patients with CPHD was selected from a group of approximately 350 patients with proven GH deficiency (GHD) (stimulated GH < 10 ng/ml) treated at our institution. Selection criteria were 1) complete GHD with 2) at least one additional pituitary hormone deficiency, 3) with or without abnormal pituitary morphology in magnetic resonance imaging (MRI).

All patients were seen at regular intervals, generally every 3 months. All auxological measurements, test procedures, and diagnostic and therapeutic management were conducted by the same team of physicians and nurses. Bone age was evaluated by the same investigator throughout the study. Height and weight were measured using precision stadiometers and scales. For calculation of height and weight SD scores (SDSs), reference ranges of central Germany were applied (12, 13), and for body mass index (BMI) national German reference data (14) were used. Bone age was determined using the method of Greulich and Pyle (15). All patients were free of any other underlying disease, such as tumor, trauma, or additional endocrine disorder unrelated to the pituitary failure. All patients or their guardians gave written informed consent to the test procedures, DNA analyses, and publication of photographs.

Endocrine function tests

All endocrine function tests were performed according to standard operating procedures based on current knowledge and literature (16). Stimulation tests were performed in the morning at 0800 h after an overnight fast. Blood samples were drawn at various time points from –30 to 180 min. All provocative agents were administered iv except glucagon, which was injected im. GH secretion capacity was assessed after stimulation with arginine (0.5 g/kg, maximum 30 g), glucagon (0.05 mg/kg; Novo Nordisk Pharma GmbH, Mainz, Germany), insulin (0.1 IU/kg Actrapid, Novo Nordisk), or GHRH (1 μg/kg, maximum 50 μg; Ferring Arzneimittel GmbH, Kiel, Germany). The cutoff level for total GHD was 5 μg/liter (for partial GHD, 10 μg/liter) for all GH stimulation tests. For glucagon and insulin tests, regular bedside monitoring of blood glucose was performed to detect hypoglycemia. The pituitary adrenal axis was evaluated using the CRH stimulation test (1 μg/kg, maximum 100 μg; Ferring) or measurement of cortisol secretion after insulin-induced hypoglycemia (cutoff level, 500 nmol/liter = 18.1 μg/dl). Other pituitary axes were evaluated using the TRH test (7 μg/kg, maximum 200 μg; Hoechst Marion Roussel, Frankfurt, Germany) for thyroid function (minimal increment of 3 mU/liter) and prolactin, and the GnRH test (50 $\mu g/m^2$ body surface area, maximum 100 μg; Hoechst) for gonadotroph function. In some instances, GHRH, TRH, CRH, and GnRH tests were combined.

When tests were performed for reevaluation after institution of replacement therapy, substitution with GH, L-thyroxine (T_4), and estradiol/testosterone was discontinued 4 wk before the stimulation tests, and for hydrocortisone 3 d before the test. No severe side effects were observed in any of the patients during or after the tests.

Biochemical analyses were routinely performed at the Institute of Laboratory Medicine, Clinical Chemistry, and Molecular Diagnosis using standard assay procedures with commercially available immunoassays and protocols. Assays are validated for inter- and intraassay variance on a regular basis. There were no substantial differences in the reference values between assays in cases where manufacturers of assays had changed, thus providing comparable data.

Genetic analyses

Analyses of the *PROP1* gene were performed in a total of 46 patients with CPHD. DNA was extracted from lymphocytes using the QIAGEN (Hilden, Germany) blood kit.

Screening for mutations within the amplified fragments was performed on a denaturing gradient HPLC (WAVE, Transgenomic, Crewe, UK) following the instructions of the manufacturer. After optimization of the method, sensitivity for detecting *PROP1* mutations was almost 100%. Samples that indicated sequence aberrations were purified with Qiaquick columns (QIAGEN) and were directly sequenced on an ABI 310 capillary sequencer (Perkin-Elmer, Norwalk, CT) using the ABI Prism Dye Terminator kit and following the cycle-sequencing protocol provided by the manufacturer.

All three coding exons of the *PROP1* gene were amplified by PCR using oligonucleotides corresponding to adjacent intronic sequences (exon 1: sense, GTC AGA GAT TCA GGG ACA CTT GG, and antisense, ATG CTT TCA GCC TCA CAC C; exon 2: sense, AGG CAC ATG TGG TCC AGC ACC, and antisense, GAT AGC ACC AAA GAA ATC TGC; exon 3: sense, CTT GTC ATT GGA GTA GGG TGT C, and antisense, CAG GAA TTC ACC ATG ATC TCC). PCR was performed using approximately 100 ng of genomic DNA and 50 pmol of each corresponding oligonucleotide. Buffer solutions and nucleotides were used according to the protocol of the *Taq* polymerase provider (QIAGEN). The PCR was carried out as follows: 30 cycles at 92 C for 30 sec, 54 C for 30 sec, and 72 C for 1 min. Gene fragments were resolved on a 2% agarose gel and stained by ethidium bromide.

Statistical analyses

Data are presented as individual values or mean ± SEM. All data are presented for n = 9 patients unless indicated otherwise. Comparisons between tests were performed using intra-individual pair-wise analysis with Wilcoxon's signed rank test. Correlation analyses were performed using Spearman's or Pearson's analysis depending on the distribution pattern of the data. A value of $P < 0.05$ was considered statistically significant.

Results

Of the 46 patients with CPHD from whom longitudinal data were available and who were analyzed for mutations in pituitary transcription factors, we identified nine patients (19.6%) with *PROP1* mutations. These nine patients have been followed for 3.0–33.2 (15.7 ± 3.8) yr by a stable team of physicians. During the observation period, all patients were under constant medical supervision for treatment and evaluation of pituitary function, including retesting with stimulation tests.

Most patients were homozygous for the 301–302delAG mutation or were compound heterozygotes for this and the 150delA mutation (Table 1). Both mutations lead to a frame shift and, consequently, loss of function of the Prop1 protein (7). Patient 2 was compound heterozygous for the 150delA deletion and another mutation, not previously described, of an intervening sequence insertion in intron 1 that affects correct splicing of the mRNA. Detailed characteristics of the patients are given in Table 1. None of the 46 patients with CPHD had mutations in the *POU1F1* or *LHX3* genes.

Patients' phenotype

Typical symptoms of CPHD with GHD include severe growth retardation with substantial bone age delay, sometimes accompanied by morphological stigmata and neonatal complications. Patients were born after uneventful pregnancies with normal birth weights and lengths (Table 2). Neonatal signs of GHD such as jaundice, hypoglycemia, or con-

TABLE 1. Patients' demographic data, *PROP1* genotypes, and replacement therapy

No.	Age (yr)	Sex	Age at first visit (yr)	Observation period	Genotype	Replacement			
						GH	T_4	S	HC
1	39.4	Female	6.2	33.2	301–302 del AG	+	+	+	+
2	31.9	Female	5.9	26.0	150 del A, IVS + 1 G → T	+	+	+	(+)
3	27.0	Male	2.5	24.6	301–302 del AG	+	+	+	(+)
4[1]	21.8	Female	5.7	16.1	301–302 del AG, 150 del A	+	+	+	+
5	17.1	Male	10.2	6.9	301–302 del AG	+	+	+	+
6	16.6	Male	5.5	11.1	301–302 del AG, 150 del A	+	+	+	+
7[1]	14.1	Male	2.3	11.8	301–302 del AG, 150 del A	+	+	+	+
8[2]	11.7	Female	3.4	8.4	301–302 del AG	+	+		+
9[2]	5.8	Male	2.8	3.0	301–302 del AG	+	+		+

Patients 4 and 7 and patients 8 and 9 are siblings belonging to two distinct families as indicated by the superscripts 1 and 2, respectively. Consanguinity was not reported for any of the parents. + Indicates substitution with GH, T_4, sex steroids (S), and hydrocortisone (HC); (+) indicates substitution, but not required regularly. Patients 8 and 9 are not yet on sex steroid replacement because of prepubertal age.

TABLE 2. Anthropometric characteristics of the patients and parents

No.	Birth weight		Birth length		Height of father		Height of mother		Target height	
	g	SDS	cm	SDS	cm	SDS	cm	SDS	cm	SDS
1	3140	0	51	0.25	172	−1.28	172	0.60	165.5	0.25
2	3520	0.25	52	0	174	−0.99	168	−0.02	164.5	−0.21
3	4250	1.046	48	−1.88	170	−1.58	152	−2.52	167.5	−0.71
4	2550	−1.88	46	−1.88	177	−0.55	166	0.34	165.0	−0.14
5	4000	0.84	53	0.25	170	−1.58	163	−0.81	172.8	−0.09
6	2520	−1.34	46	−1.565	180	−0.11	170	0.29	181.5	0.93
7	3930	0.67	51	−0.52	177	−0.55	166	−0.34	178.0	0.52
8	4570	1.88	55	1.415	182	0.19	162	−0.96	165.5	−0.06
9	4280	1.486	57	1.88	182	0.19	162	−0.96	178.5	0.58

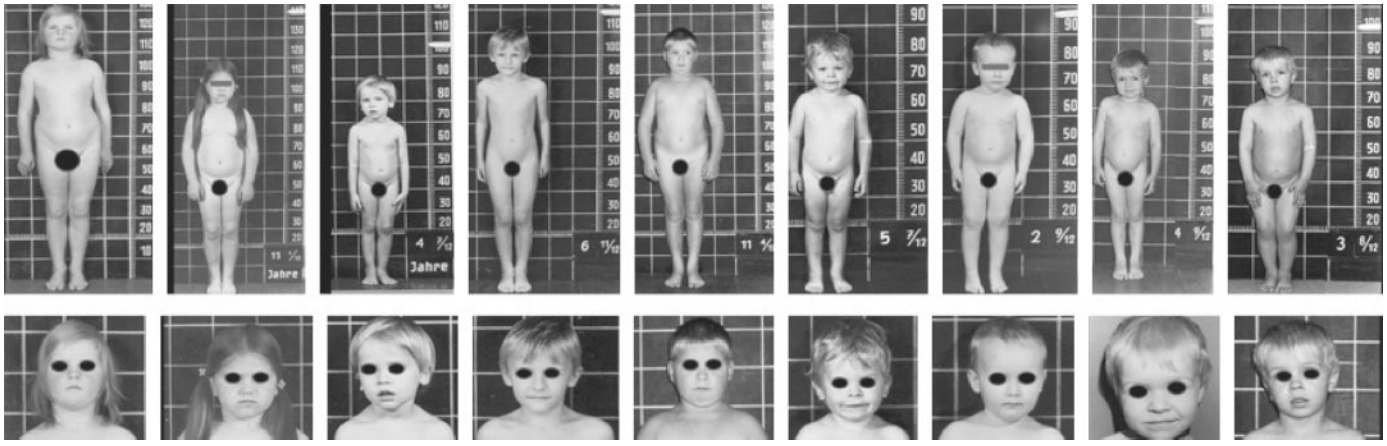

FIG. 1. Phenotype of patients. Photographs of all nine patients were taken at approximately the time of diagnosis. All patients show severe growth retardation (*arrows* indicate the 50th centile for sex and age).

vulsions were not recorded for any of the patients. Also, subsequent neuropsychomotor development was considered normal in all children.

All of our patients presented initially with profound growth retardation at a mean age of 4.9 ± 0.8 yr. At the time of initial presentation, the mean height was –3.7 ± 0.3 SDS and bone age was retarded by 3.5 ± 0.5 yr on average. The parents' heights were normal (Table 2) except for the height of the mother of patient 3 (–2.52 SDS). Consequently, patients' target heights (sex-adjusted mid-parental height) were well within the normal range (0.12 ± 0.17 SDS).

Figure 1 shows photographs of patients at approximately the time of diagnosis. In contrast to the obvious growth retardation, other features such as frontal bossing, broad nasal ridge, acromicria, and a puppet-like face were more subtle and rather infrequent. None of the boys had a micropenis, but four of the five boys had testicular maldescent with obvious cryptorchism in two boys.

Moderate truncal obesity was apparent in some patients. BMI at baseline was statistically above average although within the normal range (0.60 ± 0.20 SDS). Subsequent GH therapy for at least 1 yr did not significantly affect BMI (0.59 ± 0.16 SDS). Total serum cholesterol levels (7.6 ± 0.4 mmol/liter; normal range, 3.6–5.2) and low-density lipoprotein (LDL) levels (5.5 ± 0.5 mmol/liter; normal range, 2.1–4.9) were elevated whereas serum high-density lipoprotein (HDL) levels (1.5 ± 0.16 mmol/liter; normal range, >0.9) were low normal in all patients. In patients, who discontin-

ued GH therapy (n = 4) for reevaluation, we saw an increase in total cholesterol (7.3 ± 0.22 *vs.* 4.4 ± 0.33 mmol/liter; $P = 0.012$) and LDL (5.0 ± 0.21 *vs.* 2.9 ± 0.31 mmol/liter; $P = 0.008$) after 4 wk without GH, whereas HDL was not significantly affected. Serum IGF binding protein-3, but not IGF-I, was significantly negatively correlated with total cholesterol ($r = -0.47$; $P < 0.001$; n = 46) and LDL levels ($r = -0.49$; $P = 0.001$; n = 40) across all measurements while receiving or not receiving GH.

Pituitary morphology

All patients had at least one MRI scan performed during their clinical supervision. As can be seen in Table 3, six of the nine patients presented with a hypo- or aplastic anterior pituitary, whereas the pituitary stalk and the posterior pituitary were essentially normal. In some patients, pituitary morphology changed from a normal to hypoplastic appearance (patient 5) or enlarged pituitary (patient 9). In one boy, we saw an enlargement of the pituitary at the age of 10 yr that changed to the typical hypoplastic appearance at the age of 13 yr (Fig. 2), thus supporting the hypothesis that an enlargement of the pituitary may precede the hypoplasia.

Diagnosis of CPHD

All patients were first diagnosed with GHD based on insufficient GH responses to arginine, insulin, and/or glucagon provocation in two independent tests with GH peak levels of less than 5 μg/liter. Figure 3A depicts the average age at which substitution therapy was started, showing that GH and T_4 replacement were required in early childhood. None of the patients who were followed through the normal age range for puberty entered or completed pubertal development; consequently, all seven patients eventually received sex steroid substitution.

Follow-up evaluations

GH. Before GH replacement therapy, height measurements that were available for some patients indicate a progressive loss of height SDS (Fig. 3B). The growth patterns of patients

TABLE 3. MRI characteristics of the patients at different ages throughout the study

No.	Age (yr)	Anterior pituitary	Stalk	Posterior pituitary	Sella
1	36	Hypo-/aplastic	n	n (smaller)	n
2	28	n (decreased signal intensity)	n	n	n
3	23	Hypoplastic	Enlargement and enhancement	n	Flat
4	19	Hypo-/aplastic	n	n	n
	22	Hypo-/aplastic	n	n	n
5	9	n	n	n	n
	13	Hypoplastic	Cranial enlargement	n	Flat
	16	Hypoplastic	Cranial enlargement	n	Flat
6	12	Hypoplastic	n	n	n
	13	Hypoplastic	n	n	n
	16	Hypoplastic	Cranial enlargement	n	n
7	10	Enlarged with little gadolinium enhancement	Slight gadolinium enhancement	n	n
	13	Hypoplastic	Slight dislocation		
8	6	n	n	n	n
	11	n	n	n	n
9	3	n	n	n	n
	5	Enlarged	n	n	n

n, Normal in size and morphology.

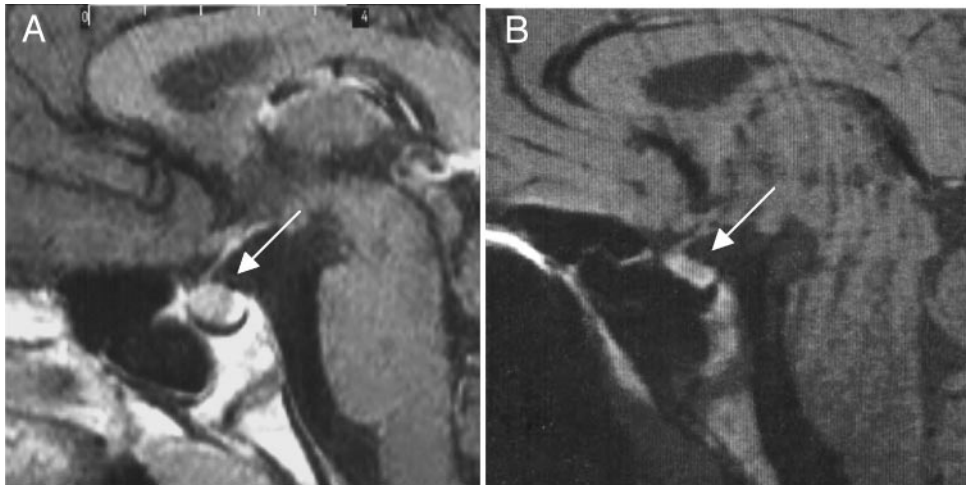

FIG. 2. Morphology of pituitary. In sequential MRIs of the same patient (no. 7) 3 yr apart, pituitary morphology changed from an enlarged pituitary at the age of 10.8 yr (A) to a hypoplastic anterior pituitary at 13.7 yr (B), indicating that enlargement as well as hypoplasia of the pituitary occur in patients with *PROP1* mutations as dynamic changes within the same patient. *Arrows* indicate the location of the pituitary.

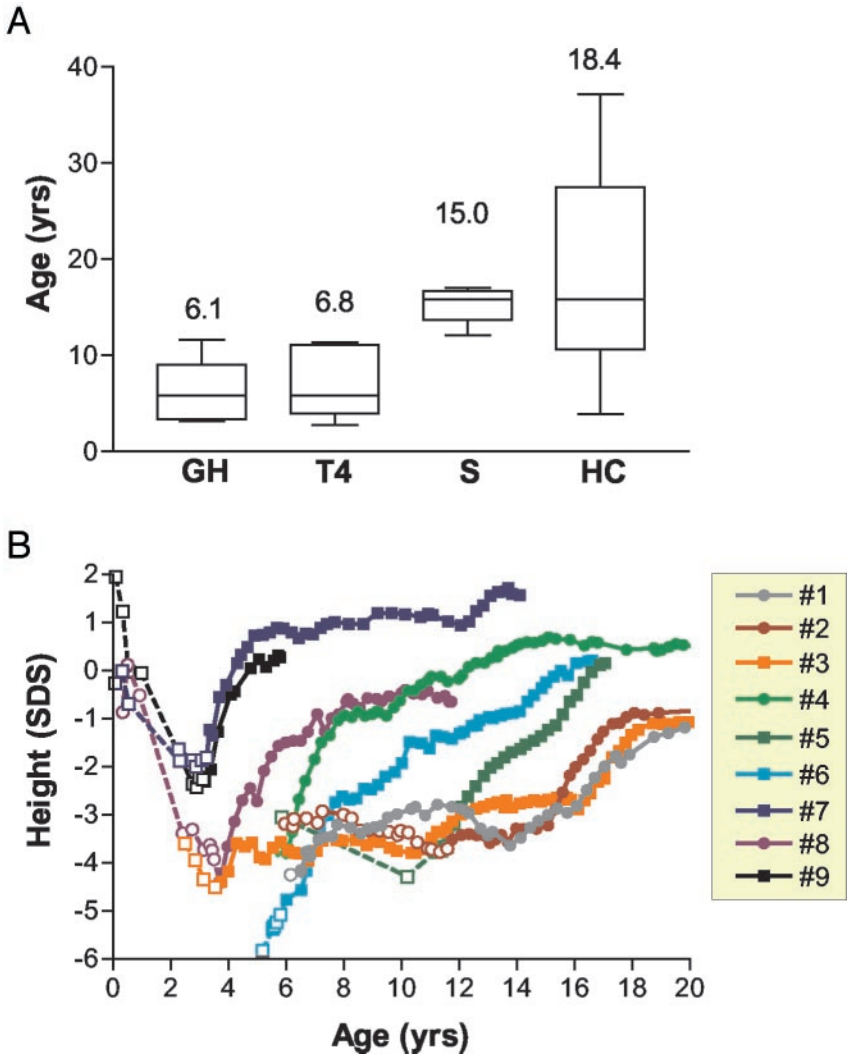

FIG. 3. A, Age at which replacement with GH, T_4, sex steroids (S), and hydrocortisone (HC) was first started. Results are mean ± SEM; n = 9 patients (for sex steroid replacement n = 7 due to prepubertal age of two patients). B, Growth patterns of patients with *PROP1* mutations. Growth curves are shown as height SDSs; *squares* represent male data, *circles* female. Data before GH substitution therapy are *open symbols* and *dotted lines*; data during GH therapy are *filled symbols*.

7, 8, and 9, starting from normal length at birth and infancy, show that height SDS rapidly declined until the time of diagnosis at approximately 4 yr of age. With GH replacement therapy, all patients showed a good growth response and eventually attained heights within the normal range (>–2.0 SDS; Fig. 3B).

During follow-up, reevaluation of GH secretion capacity revealed a further decline in the response to provocative stimuli, indicating progression of the severity of GHD (Fig. 4, A and B). The average response to GHRH, which initially provoked higher peak levels in some patients compared with arginine, insulin, and/or glucagon stimulation at the same time points (5.0 ± 1.6 μg/liter *vs.* 1.2 ± 0.2 μg/liter; $P = 0.065$; n = 5), eventually declined to undetectable levels with increasing age (Fig. 4, C and D).

TSH. TSH deficiency was diagnosed early in the clinical course based on insufficient responses in TRH stimulation tests, even though basal TSH was within the normal range initially (2.28 ± 1.1 mU/liter; n = 8; reference range, 0.85–6.5 mU/liter) with free T_4 levels only slightly decreased (6.18 ± 1.01 pmol/liter; reference range, 12.1–22.0 pmol/liter). As seen in the GH stimulation tests, TSH responses in TRH tests declined over time (Fig. 4E) with significantly lower peak levels at reevaluation (Fig. 4F). Concomitantly, there was a decrease in stimulated peak prolactin levels, which fell from 517.2 ± 99.9 mU/liter at a mean age of 7.1 ± 1.4 yr to 195.1 ± 53.0 mU/liter at 19.1 ± 3.4 yr (Fig. 3G) ($P = 0.065$; n = 5).

LH and FSH. LH and FSH levels after GnRH stimulation were low in prepubertal age and showed a tendency to decrease with increasing age. Around the age when puberty is expected, only one patient showed a subtle increase in GnRH responsiveness. However, when tested in adulthood, all patients had complete gonadotropal insufficiency with stimulated LH and FSH levels below the detection limit (Fig. 4H). The failure to develop secondary sexual signs in all patients is in accordance with these endocrine results.

Adrenal function

One of the main aims of the present study was to analyze whether adrenal insufficiency was present or developed during the course of disease in our patients with *PROP1* defects.

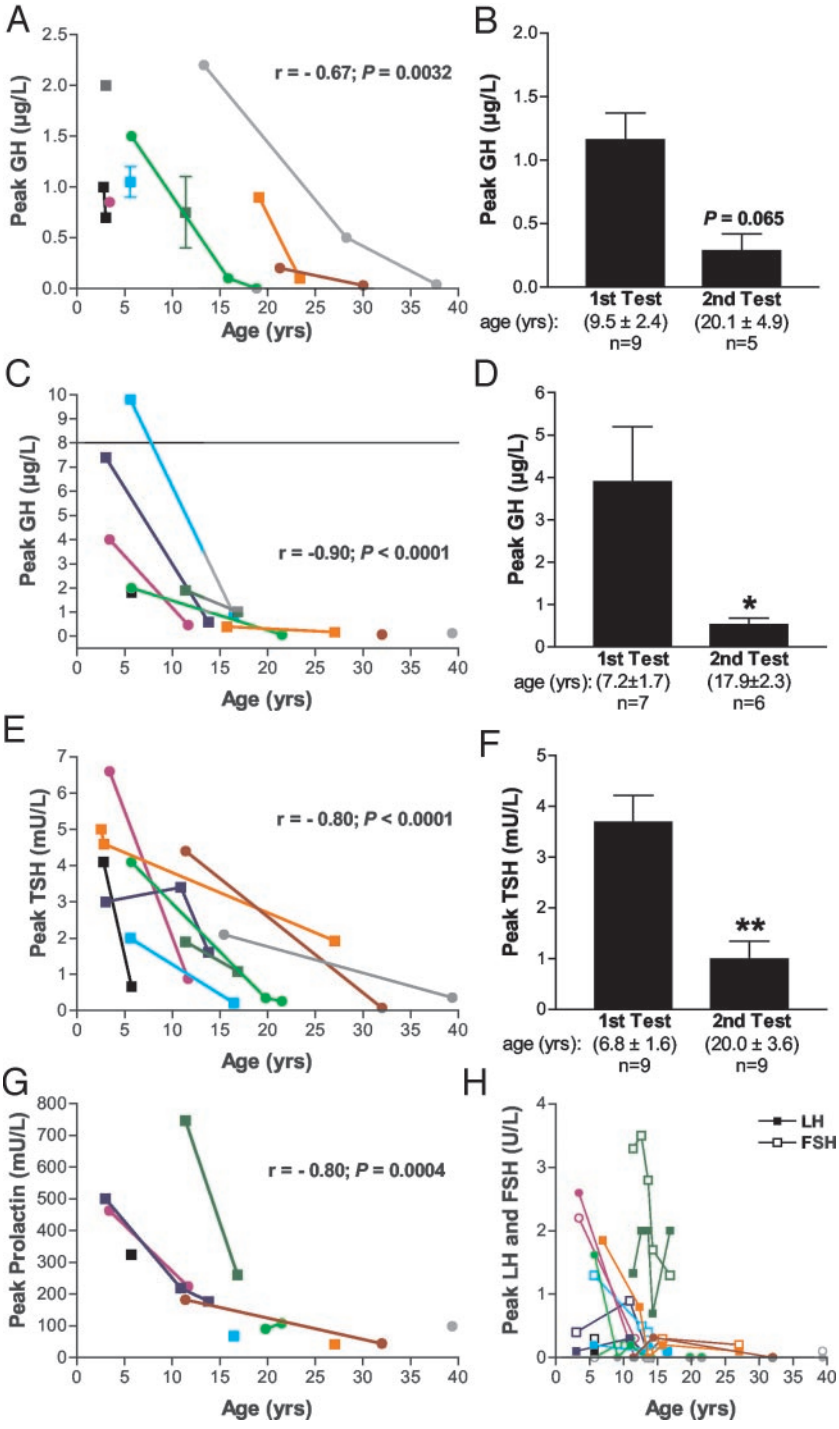

FIG. 4. Longitudinal analysis of peak levels in stimulation tests: A and B, GH stimulation tests excluding GHRH; C and D, stimulation with GHRH; E and F, TSH in TRH stimulation tests. The *left* graphs depict responses for individual patients. The *right* graphs depict mean ± SEM of peak levels in the first two sequential stimulation tests. G, Fall in stimulated prolactin levels after TRH challenge; H, course of stimulated LH (*filled symbols*) and FSH (*open symbols*) levels over time in our patients with a physiological lack of surge at prepubertal age; small increases at about the time of puberty in some patients declined to levels below detection limits afterward, suggesting central hypogonadism. *Squares* represent male data, *circles* female; correlations were assessed by Spearman's nonparametric correlation analysis. The third tests were not considered in the summarizing analysis because they were not performed in all patients (B, D, and F). Mean age and n are given below *bars*; Wilcoxon's signed rank test was applied for patients with sequential test data; *, $P < 0.05$.

When initially evaluated, none of the patients had symptoms of adrenal insufficiency, and cortisol levels were normal (15.36 µg/dl; n = 9). With increasing age, however, basal morning cortisol levels declined (Fig. 5A) and fell below the normal range in all patients at some point in their clinical course (Fig. 5A). This decline in cortisol levels was strongly and significantly negatively correlated with age in most patients where enough data for intra-individual correlation analysis were available. Basal cortisol levels at initial evaluation were higher than levels obtained just before hydrocortisone replacement was started (Fig. 5B). In accordance with this finding, most patients elicited an adequate response to CRH or insulin stimulation on first testing (Fig. 5C). As seen in other pituitary function tests, peak cortisol levels declined over time and fell below a cutoff limit of 18 µg/dl in all patients (Fig. 5C). This decline was significant when intra-individual test results were compared (Fig. 5D).

These endocrine findings correlated with clinical symptoms of adrenal insufficiency; six of our patients reported fatigue, dizziness, or increased susceptibility for colds at the

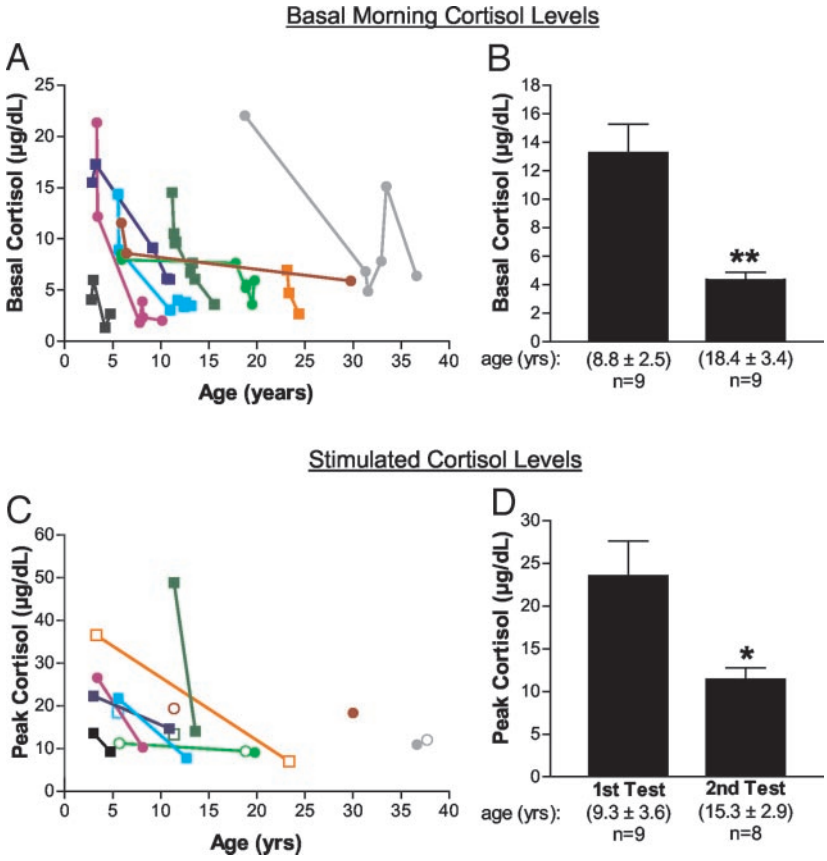

FIG. 5. Evaluation of adrenal function in patients with *PROP1* mutations. A, Decline in basal morning cortisol levels for individual patients; B, mean basal morning cortisol levels at first evaluation and at last measurement before commencing hydrocortisone replacement; C, peak levels in CRH (*filled symbols*) or insulin stimulation tests (*open symbols*); D, pair-wise intraindividual comparison. For conversion of cortisol levels to SI units, multiply cortisol in µg/dl by 27.59 to calculate nmol/liter. *, $P < 0.05$; **, $P < 0.01$ by Wilcoxon's signed rank test for initial *vs.* second test. *Squares* represent male data, *circles* female.

time when tests of adrenal function became pathological or after they had discontinued hydrocortisone therapy. As a consequence of the biochemical findings and clinical signs of adrenal insufficiency, all patients eventually required hydrocortisone therapy, with seven patients receiving a low-dose replacement on a regular basis and two patients receiving hydrocortisone only when they experienced clinical symptoms. The latter two patients had low basal morning cortisol levels (5.9 and 4.4 µg/dl, respectively) with subnormal peak levels after CRH stimulation (18.3 and 7.0 µg/dl) and intermittently experienced symptoms such as fatigue, dysphoria, apathy, and muscle cramps indicating attenuated adrenal function. In six of our nine patients, institution of cortisol replacement was necessary before the age of 20 yr.

Discussion

Hypopituitarism in patients with *PROP1* mutations typically includes deficiencies of GH, TSH, PRL, and LH/FSH. However, available data from case reports or cross-sectional studies are conflicting with respect to the time of onset and severity of pituitary dysfunction (11, 17). In the present study we performed a retrospective longitudinal analysis of nine patients in whom *PROP1* mutations were identified and characterized.

The important findings of our study are that all patients uniformly showed a decline in stimulated peak levels in anterior pituitary function tests for GH, TSH, and PRL, indicating a progressively deteriorating pattern of anterior pituitary function. This deterioration was observed for each anterior pituitary axis, even though the time points of occurrence varied. In addition, at least partial adrenal insufficiency developed in all our patients based on clinical presentation and failure of provocative tests of the pituitary adrenal axis. The strengths of the current study are 1) the longitudinal design with a long observation time, 2) consistent evaluation by the same team of investigators, and 3) a relatively large number of patients.

Our study demonstrates that patients with *PROP1* defects show progressive deterioration of pituitary function, including adrenal insufficiency, and may explain some of the inconsistencies concerning the variable degrees of pituitary dysfunction observed in single case reports.

GH and TSH deficiency are commonly the primary signs that prompt an endocrine evaluation and are generally recognized as essential findings in patients with *PROP1* defect. In contrast to patients with CPHD of other genetic origins who become symptomatic shortly after birth (7), neonates with *PROP1* defects lack perinatal signs of hypopituitarism (18) and generally have normal birth lengths. The progressive growth failure develops later in childhood (17–19), and the diagnosis is usually not made until the age of 6–7 yr (11, 19, 20); there are even reports describing ongoing growth well into adulthood in patients with *PROP1* defects (17, 21–23). Our data, showing that GH peak levels in stimulation tests decline with increasing age, support these clinical observations and underscore the progressive nature of GHD in this condition.

One metabolic consequence of GHD is the lack of lipolytic

action of GH and dyslipidemia. Even though the BMI was only slightly elevated at baseline and remained unaffected by GH therapy, we saw a significant effect of GH on total cholesterol and LDL levels. Serum lipid levels were inversely associated with IGF binding protein-3 levels, indicating a direct relationship between chronic GH action and lipid metabolism. Similar alterations in serum lipids have been reported in adults (24). Patients with GHD are at increased risk of developing cardiovascular disease, which is at least partially reversed by appropriate GH substitution (25, 26) and may profoundly affect the longevity of such patients (27); this emphasizes the life-long importance of appropriate therapy even after cessation of growth.

Similar patterns to those for GH were observed for TSH and PRL. Although all children required substitution therapy with T_4 in childhood at approximately the time when GH therapy was instituted, lactotroph function was maintained longer and some patients still had normal stimulated PRL levels when peak TSH levels were already below the diagnostic cutoff.

Whether hypogonadism is a pertinent feature in patients with *PROP1* defect has been a matter of debate. Lack of pubertal development was reported in some patients (11, 18), whereas others entered but failed to complete puberty or developed secondary hypogonadism at a later age (17, 19). Progressive failure of gonadotroph function in our patients was more difficult to demonstrate. Basal as well as stimulated LH and FSH secretion is naturally low before puberty. A subtle increase in stimulated LH and FSH was noted at pubertal age in some patients. This increase, however, did not reach concentrations typically seen in normal pubertal development and peak levels rapidly declined below the detection limit thereafter. Thus, as suggested by others (19), gonadotroph function shows a progressive decline that may clinically present as primary or secondary lack of reproductive function.

The main controversy has been whether adrenal insufficiency is a feature of *PROP1* deficiency. In contrast to what may have been expected from the biological understanding of the role of *Prop1* in embryonic pituitary development in the mouse, our data clearly indicate that adrenal insufficiency is part of this condition in humans. There are case reports demonstrating cortisol deficiency in some patients (22, 23, 28–30), whereas others did not find any signs of adrenal insufficiency (8, 17, 19, 21). These conflicting data may reflect variations in study design; most studies relied on cross-sectional data obtained at a single time point and therefore cannot evaluate the potential development of adrenal insufficiency later in the clinical course. However, the longitudinal design of our study offers some explanation of these discrepancies, in that basal and stimulated cortisol levels can be normal in childhood but decline later. This decline was accompanied by mild clinical symptoms in most of our patients, even though the cortisol response to CRH or insulin stimulation was not completely abolished. This indicates that some residual capacity of the pituitary adrenal axis is preserved and may also explain the rather mild clinical phenotype. Therefore, adrenal insufficiency needs to be considered and adrenal function must be carefully monitored because these patients are at risk of developing adrenal failure. We recently reported on an adult patient with life-threatening adrenal crisis who had not been diagnosed with adrenal insufficiency before, and a *PROP1* mutation was identified in the subsequent diagnostic work-up (29). Also, the youngest patient in the present study had been hospitalized with symptoms of cerebral convulsions and electrolyte imbalance, consistent with adrenal crisis after he discontinued hydrocortisone replacement because of noncompliance. Even though, in the literature, the onset of adrenal insufficiency was usually reported to occur in adulthood (22, 23, 28, 31), our study provides evidence that it can emerge at any age in patients with *PROP1* defect, even in childhood. It also needs to be considered that the time of diagnosis does not necessarily coincide with the actual time when adrenal function deteriorates, particularly if patient management does not include regular monitoring of adrenal function.

The exact genotype of a patient with a *PROP1* mutation also needs to be considered in the context of the phenotypic variability, because the degree of loss of function differs between the reported mutations. For instance, the R120C mutation, for which spontaneous puberty has been reported, leads to a protein with 12% retained function, whereas other mutations completely abolish DNA binding and transcriptional activity, as reported for the most frequently occurring 301–302delAG and 150delA mutations (4, 10). On the other hand, a considerable phenotypic variability was reported for the same mutation within one family (17, 22). However, it appears that the variability in the clinical phenotype of the patients at the time of diagnosis is mainly due to differences in the age of onset and the time course of the deterioration of anterior pituitary function, which is distinct in the individual patients. Thus, due to this progressive pattern, the extent of interindividual variability is highest in childhood and decreases with the age of patients when all patients have experienced complete anterior pituitary insufficiency.

It is also not clear how the endocrine phenotype of patients with *PROP1* mutations correlates with pituitary morphology. The anterior pituitary was hypoplastic in six of our nine patients. The earliest age at which hypoplasia was detected was 12 yr. Because our youngest patients are not yet at this age, the prevalence of hypoplasia is expected to increase. It is also known that pituitary morphology can change during follow-up in these patients (32), with a transient enlargement of the anterior pituitary that is followed by involution leading to a hypoplastic gland, which we also detected in one patient (22, 30). However, we and others did not find any association of hypoplasia with the endocrine findings. The morphology of the stalk and posterior pituitary was essentially undisturbed, which appears to be a typical feature of CPHD of genetic origin (33), in contrast to patients with idiopathic GHD who frequently present with an ectopic posterior pituitary and abnormal pituitary stalk (33).

The pathophysiological mechanisms that cause progressive abolition of anterior pituitary hormone production are not immediately obvious. It has become clear that these mechanisms, particularly with respect to corticotroph function, appear to be complex and are not simply restricted to the failure in embryonic development of the cell lineage. One possible explanation may be progressive apoptosis and secondary decompensation of specific pituitary cell lineages. It

is conceivable that the role of *PROP1* as an important factor in the embryonic differentiation process may further extend to the maintenance of pituitary cell lineages and pituitary hormone production later in life. This hypothesis is supported by the fact that *PROP1* expression is not completely switched off during embryonic development but has been demonstrated to persist in adult pituitaries and pituitary tumors (34, 35).

Even though from current knowledge it appears unlikely that deficiency of the *PROP1* transcription factor causes ACTH deficiency directly, the clinical finding of an impaired pituitary adrenal axis suggests that *PROP1* has some role in differentiation or viability of corticotroph cells. It may well be that the progressive lack of important paracrine signals from the (deceased) surrounding pituitary cells induces progressive cell death or apoptosis of the corticotroph cells. The clinical finding that adrenal insufficiency occurs after the other anterior pituitary axes have succumbed is in line with this hypothesis. On the other hand, patients with *POU1F1* mutations, which may also show some progressiveness in pituitary dysfunction (36) characterized by GH, TSH, and PRL deficiency, have not been reported to develop adrenal failure.

Finally, pituitary masses (22, 37) as well as pituitary hypotrophy (38) were described as sequential events within the same patient (12, 13). Persistence of earlier pituitary transcription factors that are not properly down-regulated due to diminished Prop1 action may induce growth of undifferentiated tissue leading to the formation of pituitary masses. The accumulation of such fibrous material (39, 40) may affect the viability of residual functional cells.

In conclusion, the detailed, long-term, follow-up analysis of the endocrine phenotype of patients with *PROP1* defect provides evidence that anterior pituitary function deteriorates progressively and that adrenal insufficiency is a pertinent feature of this condition. Considering the deterioration of all anterior pituitary axes observed in this study one may well refer to PROP1 (equivalent to PROP I) deficiency as a disorder of progressive pituitary insufficiency. These results have important clinical implications for patients with *PROP1* mutations in that such patients face a risk of developing adrenal insufficiency in childhood or adolescence, and the time point of occurrence is not predictable. Considering that *PROP1* gene defects account for the majority of inherited CPHD (6), clinicians should carefully monitor the clinical course of patients with combined deficiency of GH and TSH for the emergence of adrenal insufficiency or hypogonadism. Likewise, with increasing availability of molecular biology tools, an early diagnosis of the precise genetic defect is worthwhile because it has important clinical implications concerning diagnosis, prognosis, and clinical management of the patients.

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