Recombinant Human Growth Hormone Replacement in a Japanese Man with a Novel PROP1 Gene Mutation (R112X)

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Recombinant Human Growth Hormone Replacement in a Japanese Man with a Novel *PROP1* Gene Mutation (R112X)

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Abstract Congenital combined pituitary hormone deficiency (CPHD) is associated with deficiencies of anterior pituitary hormones. *PROP1* gene mutations are often responsible for CPHD, but few such cases have been reported in Japan. This study describes a 37-year-old Japanese man with CPHD, treated with hydrocortisone, testosterone, and L-thyroxine, who was evaluated for adult growth hormone deficiency (GHD). Gene analysis revealed a previously unknown *PROP1* mutation (R112X). After 10 months of recombinant human growth hormone (rhGH) administration, cortisol and urinary free cortisol levels were significantly lower than before therapy. This case underscores the importance of reassessing hypothalamic-pituitary-adrenal axis function in GHD patients, especially those with a *PROP1* mutation, during rhGH therapy.

Key words : PROP1 mutation, Growth hormone deficiency, Growth hormone Replacement, Hypothalamic-pituitary-adrenal axis

Introduction

Adult growth hormone deficiency (AGHD) results in loss of lean body mass and bone mineral density (BMD), increased visceral adiposity, an adverse metabolic profile, and elevated cardiovascular risk[1)2)]. Growth hormone (GH) replacement ameliorates these conditions[3)]. GHD is frequently associated with congenital combined anterior pituitary hormone deficiency (CPHD), which is characterized by impaired production of GH and one or more of the other anterior pituitary hormones. *PROP1* gene mutations appear to frequently be responsible for CPHD[4)], particularly in Caucasians, but few cases have been reported in Japan[5)6)]. We describe a 37-year-old Japanese man with AGHD who was treated with recombinant human GH (rhGH). Molecular analysis of *PROP1* identified an R112X (Arg 112 Ter) mutation. Recent studies indicate that impaired GH secretion may mask deficiencies of other pituitary hormones[7)8)]. Hence, rhGH replacement therapy was evaluated in this patient, focusing especially on the hypothalamic-pituitary-adrenal (HPA) axis.

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Case report

Patient (Table 1, Fig. 1)

The patient had been born at term after a normal pregnancy to Japanese parents. The delivery and postnatal course were uncomplicated. Short stature became evident at the age of 3 years, but was not treated at the time. At age 8, he was hospitalized for treatment of short stature (-6.1SD score). His bone age was delayed by 4 years. Arginine or insulin tolerance tests revealed GH deficiency and his serum thyroid-stimulating hormone (TSH) level was normal with a blunted response to thyrotropin releasing hormone (TRH). GH treatment was initiated. At age 11, hypothyroidism was diagnosed and he was admitted to another hospital. His baseline and

Table 1 Hormone concentrations obtained in different provocation tests

Age	8 yrs	11 yrs	37 yrs
Arginine (Peak)			
GH (ng/mL)	2.3		
Glucagon (Basal/Peak)			
GH (ng/mL)			< 0.03/< 0.03
GRH (Basal/Peak)			
GH (ng/mL)			< 0.03/0.04
GHRP-2 (Basal/Peak)			
GH (ng/mL)			< 0.03/0.04
Insulin (Peak)			
Glucose (mg/dL)	29		
GH (ng/mL)	1.4		
Methyrapone (Peak)			
ACTH (pg/mL)		33.6	
ACTH (Basal/Peak)			
Cortisol (nmol/L)			11.5/20
CRH (Basal/Peak)			
ACTH (pg/mL)			33.6/80.4
Cortisol (μg/dL)			10/15.2
TRH (Basal/Peak)			
TSH (mIU/L)	2.4/10.4	< 2.0/< 2.0	< 0.05/< 0.05
T3 (ng/mL)		< 0.5/ND	
T4 (μg/dL)		< 0.5/ND	
PRL (μg/L)		< 3.0/< 3.0	1.97/3.03
GnRH (Basal/Peak)			
LH (IU/L)		< 2.0/< 2.0	< 0.1/< 0.1
FSH (IU/L)		2.2/2.2	< 0.1/< 0.1

ND : Not done.
Provocation tests at the age 37 were performed before patient received 75 μg per day of levothyroxine, 5 mg per day of hydrocortisone in the morning.

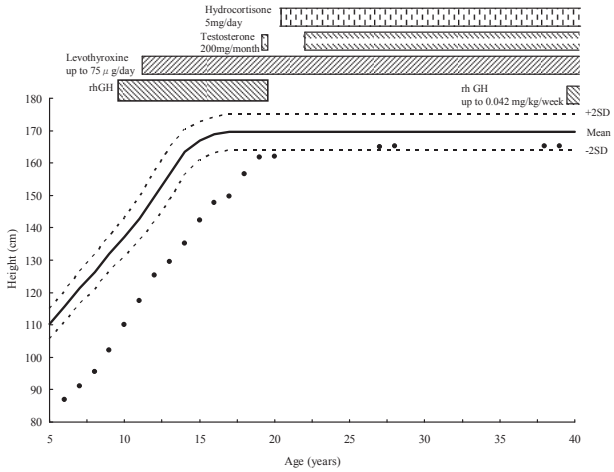

Fig. 1 Summary of the previous treatment and growth chart of the patient (close circle).
rhGH; recombinant human growth hormone

peak levels of serum TSH, triiodothyronine (T3), thyroxine (T4), and prolactin (PRL) were very low in response to TRH. In addition, his baseline and peak levels of serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) were very low in response to luteinizing hormone-releasing hormone (LHRH). However, with metyrapone stimulation adrenocorticotropic hormone (ACTH) was within normal range. He was diagnosed with combined GH, TSH, LH, FSH, and PRL deficiencies, and levothyroxine treatment was initiated. At age 18, testosterone treatment was started. By age 19, his height was 161.8 cm (bone age 16), but GH and testosterone administrations were discontinued due to noncompliance. Hydrocortisone treatment was initiated at age 20 because of general fatigue, and testosterone was restarted at age 23. In 2007, at age 37, he was admitted for evaluation of AGHD (Table 2). His height was 166 cm, weight 55.5 kg (BMI : 20.2 kg/m^2), and abdominal circumference 82 cm. He was treated with 75 μg/day levothyroxine, 5 mg/day hydrocortisone, and 200 mg/month testosterone. The serum testosterone concentration was in the normal range after replacement therapy with testosterone enanthate. Serum thyroid hormone levels were also within low-normal ranges. Insulin-like growth factor-1 (IGF-1) hormone level and IGFBP-3 level were below normal. Magnetic resonance imaging of the pituitary revealed marked hypoplasia of the anterior gland, with the sella appearing empty, and a normal pituitary stalk (Fig. 2). BMD of the lumbar spine (L2-4) was 0.746 g/cm^2 by dual energy X-ray absorptiometry (DEXA). The patient's visceral fat area was 86.2 cm^2 by cross-sectional computed tomography (CT) obtained at the umbilicus.

Stimulation tests

The combined pituitary stimulation test was performed employing simultaneous intravenous administrations of 100 μg LHRH, 500 μg TRH, 100 μg GH-releasing factor (GRF), and 100 μg

corticotropin releasing hormone (CRH). Blood samples were collected before and at 30, 60, 90, and 120 minutes after the injection of the hypothalamic neuropeptides, for measurement of serum LH, FSH, TSH, PRL, GH, ACTH, and cortisol. In addition, an adrenal stimulation test was performed by intravenously administering 100 μg ACTH, and blood samples were collected before and at 30 and 60 minutes after the injection. Furthermore GH stimulation test was performed by administration of 100 μg GHRP-2 or 1mg glucagon and blood samples were collected before and at 15, 30, 45, and 60 minutes or 30, 60, 90, 120, 150 and 180 minutes, respectively, after the injection. Basal serum concentrations of GH, LH, FSH, and TSH were low, while ACTH and PRL levels were normal (Table 2). GH, LH, FSH, and TSH responses were absent, that of PRL was attenuated relative to normal, and ACTH and cortisol responses were normal (Table 1).

Table 2 Basal hormone concentrations at 37 years old

GH	< 0.3 ng/mL	TSH	< 0.003 μU/mL
IGF-1	23 ng/mL	fT4	0.93 ng/dL
IGFBP-3	0.71 μg/mL	ACTH	33.6 pg/mL
LH	< 0.1 mIU/mL	Cortisol	10.0 μg/dL
FSH	< 0.1 mIU/mL	PRL	1.97 ng/mL
Testosterone	5.41 pg/mL	ADH	1.6 pg/mL

Hormone concentrations were measured under 200 mg per month of testosterone, 75 μg per day of levothyroxine, and 5 mg per day of hydrocortisone.

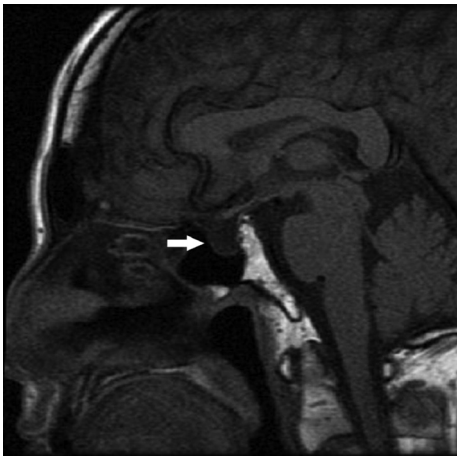

Fig. 2 Sagittal image obtained by MRI showing the hypoplastic pituitary gland of the patient (arrow).

Genomic analysis of the PROP1 gene

Genomic DNA from the patient's peripheral blood was extracted using a DNA blood kit (Qiagen, Hilden, Germany). All three exons of the *PROP1* gene were amplified by PCR, using the primers as follows, 5'-TGCCTGCACCTACA CACACATTCAG-3' and 5'-TTTCTTGTCTTT TCACGAGGGCCGC-3'. PCR was performed in a final volume of 25 μL containing 10 mM Tris-HCl (PH8.3), 50 mM KCl, 1.5 mM MgCl2, 200 nM of each dNTP, 1 mM of each primer, 100 ng genomic DNA, and 5 units of LA Taq polymerase (Takara, Shiga, Japan). Amplifications were performed with 35 cycles, as follows : 94℃ for 1 min and 65℃ for 5 min. The PCR products were purified using Montage PCR Centrifugal Filter Devices (Millipore, Bellerica, MA) and subsequently sequenced. Sequencing was performed using a BigDye Terminator Cycle Sequencing Kit and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Comparison with the wild-type sequence revealed the patient to be homozygous for a single base-pair substitution (C to T) at codon 112 (CGA > TGA; R112X), resulting in an Arg to stop codon

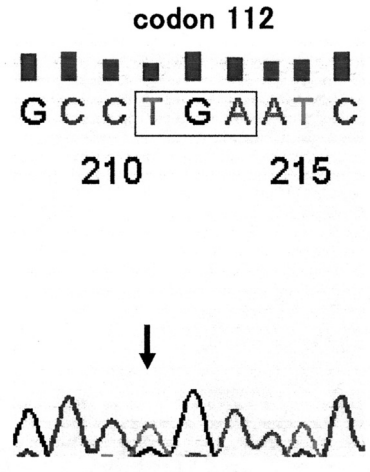

Fig. 3 Direct sequencing of the coding exons of the PROP1 gene. Comparison with the wild-type sequence revealed the patient to be homozygous for a single base-pair substitution (C toT) at codon 112 (CGA > TGA; R112X).

substitution at position 112 (R 112X) (Fig. 3). Unfortunately, a pedigree analysis could not be performed because the patient and his family lived far from our hospital.

recombinant human growth hormone (rhGH) treatment

The treatment was adjusted to reach the age-matched normal IGF-1 level (67-318 ng/ml). The rhGH dose was raised from 0.021 mg/kg/week to 0.042 mg/kg/week.

rhGH treatment results

At 10 months after starting rhGH therapy, there were no significant changes in metabolic indices. Visceral fat was reduced from 86.2 cm^2 to 58.1 cm^2 on CT, and BMD of the lumbar spine (L2-4) had increased from 0.746 g/cm^2 to 0.764 g/cm^2 by DEXA. Intriguingly, circadian cortisol secretions, as well as the urinary free cortisol (UFC) concentration, were significantly lower after starting rhGH therapy (Table 3a). Similarly, responses to provocative tests revealed rhGH therapy to have significantly reduced basal and peak serum cortisol levels as compared to before treatment (Table 3b). Central hypoadrenalism was evident after rhGH therapy but without signs of adrenal insufficiency at present.

Table 3a Circadian secretion of cortisol before and after rhGH therapy

	ACTH (pg/ml)		Cortisol (μg/dl)	
Time of day	Before	After	Before	After
0900h	22.2	29.4	26.3	10.4
1700h	20.7	18.7	8.3	3
2100h	11.4	16.9	3.2	1.8

Hormone concentrations were measured after patient received 5 mg per day of hydrocortisone in the morning in both of before and after rhGH therapy.

Discussion

The *PROP1* gene is a paired-like homeobox gene mapped to chromosome 5q35 in humans. This gene encodes a 233-amino-acid nuclear protein that contains a C-terminal trans-activation domain and a paired-like homeodomain with three putative αhelices[9]. Mutations in the more recently recognized *PROP1* gene are rather frequently detected as the genetic basis for CPHD, particularly in Middle and Eastern Europe and North America, but to our knowledge in the English literature, few cases have been reported in Japan[5)6)]. In the present Japanese case, we obtained clinical and hormonal data spanning the period from childhood into young adulthood, and molecular analysis of *PROP1* identified an R112X (Arg 112 Ter) mutation. Several *PROP1* gene mutations have been identified as structurally affecting the paired-like DNA-binding domain of the *PROP1* protein molecule[4]. R112X is a newly identified nonsense mutation. Its translation disrupts the paired-like homeodomain that is required for DNA interaction with the *PROP1* protein, leading to complete loss of the mutant protein's DNA binding activity.

PROP1 gene mutations are associated with deficiencies of GH, TSH, FSH, LH, PRL, and ACTH. The secretions of these pituitary-derived hormones all decline gradually with age. In our case, GH replacement therapy had been administered from age 8 to 19 years, and rhGH therapy was restarted for treatment of severe AGHD detected in our hospital. The administrations of 75 μg/day levothyroxine, 5 mg/day hydrocortisone, and 200 mg/month testosterone

Table 3b Results of CRH test and ACTH test, and UFC before and after rhGH therapy

	CRH test				ACTH test		UFC (μg/day)
	ACTH (pg/mL)		Cortisol (μg/dL)		Cortisol (μg/dL)		
	Basal	Peak	Basal	Peak	Basal	Peak	
Before	33.6	80.4	10	15.2	11.5	18	26.4
After	32.9	57.5	4.2	7.2	4.3	8.4	< 8.9

CRH test or ACTH test were performed before patient received 5 mg per day of hydrocortisone in the morning in both of before and after rhGH therapy.

are ongoing.

Favorable effects, such as reduced visceral fat and increased BMD of the lumbar spine, were observed after only 10 months of rhGH therapy in this case. However, rhGH replacement was also found to have a major effect on the HPA axis. The patient showed a significant decrease in circadian secretions of cortisol and UFC, and a blunted cortisol response to provocative tests. Giavoli et al.[10] reported that serum cortisol levels and UFC concentrations were significantly lower on, as compared to before rhGH therapy, whereas mean serum corticosteroid binding globulin levels did not change in GHD patients. Furthermore the serum cortisol peak after either an ACTH or an insulin tolerance test was also lower on rhGH therapy. In the current case, the CRH test indicated that sufficient ACTH expression was maintained after starting rhGH therapy, which suggested that ACTH was not associated with the serum cortisol and UFC reductions. There have been studies showing that GH influences peripheral cortisol metabolism by reducing the activity of enzyme 11 beta hydroxysteroid dehydrogenase type1 (HSD1), which converts inactive cortisone to active cortisol in GHD patients[11]. In fact, it has been observed that 11 beta HSD1 activity is decreased in patients with GHD during rhGH therapy[12] and in siblings with acromegaly[13]. Furthermore, Gelding et al[14] reported that 4 months of GH replacement in patients with hypopituitarism, who were also ACTH deficient, resulted in a significant reduction of 11 beta HSD1 and an increase 11 beta HSD type 2 mRNA expression, with increased IGF-1, in subcutaneous fat deposits. These studies suggested that GH (IGF-1) deactivates glucocorticoid through its effect on 11 beta HSDs. Therefore, regardless of a cause to become GHD, evaluation of HPA axis should be important for all GHD patients during rhGH replacement.

Based on the available reports, it is now evident that in patients with *PROP1* gene defects, the degrees of hormone deficiencies and the age at onset of such deficiencies are variable, with no clear correlations between phenotype and genotype[15]. The development of ACTH insufficiency could be due to progressive corticotroph apoptosis[16]. In fact, a progressive late-onset ACTH deficiency, associated with a risk of shock due to acute corticosteroid insufficiency, has been reported only for CPHD patients with *PROP1* mutations[17]. Furthermore, low GH and IGF-1 levels, likely enhancing the conversion of cortisone to cortisol, may mask central hypoadrenalism. It is suspected that GHD and rhGH therapy exert complex influences on the timing and degree of hypoadrenalism in patients with *PROP1* mutations. Hence, we administer low-dose hydrocortisone replacement therapy (5 mg/day) continuously in order to prevent adrenal insufficiency in conditions of severe physical or psychological stress.

In contrast, cortisol is essential for adipocyte differentiation, adipogenesis, and visceral fat distribution[18]. A lower level of active glucocorticoids may result in lower lipoprotein lipase activity[19], reduced differentiation of pre-adipocytes into mature adipocytes[20] and may change the production of adipokines[21]. Therefore, rhGH treatment for GHD may favorably change body composition, with increased lean body mass and loss of fat mass, as in the current case.

Therefore, careful long-time evaluations of HPA axis function and physical condition GHD patients with *PROP1* mutations on rhGH therapy are required, and reassessment of glucocorticoid doses during rhGH therapy is necessary to ensure that adequate replacement is achieved.

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（和文抄録）

未知の R112X 変異を伴った *PROP1* 遺伝子異常症で，成長ホルモン補充療法を開始した日本人男性の一例

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先天性下垂体ホルモン複合欠損症は，下垂体前葉ホルモンが先天性にいろいろな欠損を伴う疾患である．*PROP1* 遺伝子異常症はしばしば先天性下垂体ホルモン複合欠損症の原因となりうるが，日本人患者の報告は非常に少ない．本症例は 37 歳日本人男性の *PROP1* 遺伝子異常症であり，ハイドロコートン，テストステロンおよびチラージン S の補充療法を以前から受けている．今回精査の結果，重症成人成長ホルモン分泌不全症が判明し成長ホルモンの補充を開始した症例である．遺伝子解析にて R112X（Arg 112 Ter）と今までに報告のない変異を伴った *PROP1* 遺伝子異常症であることが判明した．ところで成長ホルモン補充開始後 10 カ月において，血中コルチゾール，尿中コルチゾールが補充前と比較して有意な低下を認めた．成長ホルモンの補充を開始した *PROP1* 遺伝子異常症において，視床下部―下垂体―副腎系の厳重な経過観察が重要であることが示唆された．