

Description of a Brazilian Patient Bearing the R271W *Pit-1* Gene Mutation

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ABSTRACT

The pituitary-specific transcription factor Pit-1/GHF-1 is responsible for pituitary development and expression of somatotrophs and lactotrophs as well as hormonal regulation of the prolactin (PRL) and thyrotropin (TSH) β genes by thyrotropin-releasing hormone (TRH) and cyclic adenosine monophosphate (cAMP). *Pit-1* gene mutations result in complete growth hormone (GH) and PRL deficiencies and variable degrees of TSH deficiency, producing the clinical syndrome of combined pituitary hormone deficiency (CPHD). Several cases of mutations in the *Pit-1* gene have been reported; the most common one is a sporadic mutation altering an arginine (R) to a tryptophan (W) in codon 271, in one allele of the *Pit-1* gene. We describe a case of a 38-year-old woman, born to consanguineous parents, presenting with growth failure and hypothyroidism. Growth failure was noted from early infancy, whereas hypothyroidism was only apparent from adolescence. She had almost undetectable GH and PRL levels and an inappropriate low TSH for very low triiodothyronine (T₃) and thyroxine (T₄) levels, while the remaining pituitary evaluation was normal. The pituitary gland was hypoplastic by magnetic resonance imaging. A point mutation in exon 6, monoallelic, causing a C to T substitution that changes amino acid 271 from Arg (R) to Trp (W) was identified. Children with Pit 1 mutations and delayed onset of hypothyroidism may be initially diagnosed as isolated GH deficiency.

INTRODUCTION

PIT-1, ALSO KNOWN AS GHF-1, is an anterior pituitary-specific transcription factor that regulates the expression of growth hormone (GH), prolactin (PRL) and thyroid-stimulating hormone- β (TSH β) genes. It plays a major role in differentiation, proliferation and hormonal production of somatotrophs and lactotrophs (1–5). During development, *Pit-1* gene expression precedes GH and PRL gene expression in these cells (2). Pit-1 is important for hormonal regulation of the TSH β gene by thyrotropin-releasing hormone (TRH) and cyclic adenosine monophosphate (cAMP) (6,7) but it is not limiting for cell-specific expression of this gene in thyrotrophs (8). Moreover, a thyrotroph-specific variant of Pit-1 (Pit-1 T) is required for TSH β promoter stimulation (9).

Mutations of the *Pit-1* gene have been found in dwarf mouse strains displaying hypoplasia of GH, PRL, and TSH-secreting cells, demonstrating the importance of Pit-1 for anterior pituitary development (10). In humans, the *Pit-1* gene locus is assigned to chromosome 3p, and it contains 6 ex-

ons and 5 introns (11,12). The protein contains 291 amino acids and comprises three main functional domains: POU-homeo, POU-specific, and transactivation domains (3–5). Human *Pit-1* mutations leading to a syndrome of combined pituitary hormone deficiency (CPHD) have been described, including dwarfism due to complete GH deficiency, PR, deficiency, and variable degrees of TSH deficiency (13–23).

We describe the case of a 38-year-old woman with CPHD, in whom analysis of the *Pit-1* gene revealed a point mutation in codon 271. The delayed onset of hypothyroidism in patients with such mutations suggest that some individuals initially identified as isolated GH deficiency may have *Pit-1* gene mutations.

MATERIALS AND METHODS

Endocrinological and neuroradiological investigations

GH was measured by immunoradiometric assay (Bio-clone Australia Pty Ltd, Marrickville, Australia), with intra- and interassay coefficients of variation of 1.4% and

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3.2%, respectively, at 5 mIU/L. The sensitivity of the assay was less than 0.2 mIU/mL ($\text{mIU/mL} \times 0.5 = \text{ng/mL}$). IGF-I (Insulin-Like Growth Factor-I) and IGFBP-3 (Insulin-Like Growth Factor Binding Protein-3) were measured by radioimmunoassay. TSH was measured by immunoradiometric assay (magnetic solid phase; MAIAclone kit, Rome, Italy), with intra-assay coefficient of variation of 3.1% at 1.16 $\mu\text{IU/mL}$, and interassay coefficient of variation of 3.2% at 1.13 $\mu\text{IU/mL}$. The detection limit of the assay was between 0.02 and 0.04 $\mu\text{IU/mL}$ ($\mu\text{IU/mL} = \text{mIU/L}$). Total thyroxine (T_4) was measured by solid-phase radioimmunoassay (Coat-A-Count kit, Los Angeles, CA), with intra-assay coefficient of variation of 3.8% at 2.4 $\mu\text{g/dL}$, and interassay coefficient of variation of 14.5% at 2.3 $\mu\text{g/dL}$ ($\mu\text{g/dL} \times 12.87 = \text{nmol/L}$). Total triiodothyronine (T_3) was measured by solid-phase radioimmunoassay (Coat-A-Count kit), with intra-assay coefficient of variation of 8.9% at 56 ng/dL, and interassay coefficient of variation of 10.0% at 59 ng/dL ($\text{ng/dL} \times 0.01536 = \text{nmol/L}$). PRL was measured by IRMA (magnetic solid phase; MAIAclone), with intra-assay coefficient of variation of 3.2% at 6.2 ng/mL, and interassay coefficient of variation of 6.0% at 6.7 ng/mL. The detection limit of the assay was 0.3 ng/mL ($\text{ng/mL} = \mu\text{g/L}$). Luteinizing hormone (LH) was measured by IRMA (magnetic solid phase; MAIAclone kit), with intra-assay coefficient of variation of 10% at 1.1 mIU/mL, and interassay coefficient of variation of 12.5 mIU/mL at 1.2 mIU/mL ($\text{mIU/mL} = \text{IU/L}$). Follicle-stimulating hormone (FSH) was measured by IRMA (magnetic solid phase; MAIAclone kit), with intra-assay coefficient of variation of 3.5% at 5.42 mIU/mL, and interassay coefficient of variation of 5.4% at 4.98 mIU/mL ($\text{mIU/mL} = \text{IU/L}$). Cortisol was measured by radioimmunoassay (Coat-A-Count kit), with intra- and interassay coefficients of variation of 2.5% and 6.3%, respectively, at 5 $\mu\text{g/dL}$ ($\mu\text{g/dL} \times 27.59 = \text{nmol/L}$). Insulin was measured by solid-phase radioimmunoassay (Coat-A-Count kit), with intra- and interassay coefficients of variation of 6.0% and 10.0%, respectively, at 16 $\mu\text{IU/mL}$ ($\mu\text{IU/mL} \times 7.175 = \text{pmol/L}$).

The plasma GH response was studied with two provocative tests: clonidine stimulation (4 $\mu\text{g/kg}$, orally) and in-

sulin-induced (0.1 and 0.15 IU/kg, intravenously) hypoglycemia.

A TRH test (200 μg , intravenously) was performed with measurements of TSH and PRL.

LH and FSH were measured in response to luteinizing hormone-releasing hormone (LHRH) (100 μg , intravenous, Relisorm® L, Serono, Mexico) and cortisol was determined in response to insulin-induced hypoglycemia.

An overnight dexamethasone suppression test (1 mg, orally, at midnight) with measurement of cortisol was performed to exclude Cushing's syndrome. An oral glucose tolerance test (glucose 75 g) with measurement of glucose and insulin was also performed.

The sellar region was scanned by magnetic resonance imaging (MRI).

Genomic analysis of the Pit-1 gene

The polymerase chain reaction (PCR) was used to amplify exons 5 and 6 from the Pit-1 gene. Oligonucleotide primer pairs (16) amplified regions that included each exon and exon-intron boundaries. The PCR products were purified using gel purification kits (Qiagen, Inc, Chatsworth, CA). Two sequencing reactions were performed with Applied Biosystems Incorporated's Dye Terminator *Taq* FS kits. The Sequencer Program (Gene Codes Corporation, Ann Arbor, MI) was used to compare the sequencing with the Genbank sequence.

RESULTS

A 38-year-old woman seen by the Endocrine Unit of the Clinical Hospital of the Federal University of Parana since 1971, because of growth failure and hypothyroidism was studied.

She was born to consanguineous parents of normal stature. A normal vertex delivery at full-term gestation occurred at home, without major complications. There were no birth records, but according to her mother, she was rather small. Growth failure was noted from early infancy and height was more severely affected than weight. Psy-

TABLE 1. STIMULATION TESTS

Test	Substance assayed and baseline reference values		Time (min)					
			0	20	30	40	60	120
TRH stimulation	TSH	(0.43–3.8 mIU/L)	1.96		2.29		2.00	
	T_4	(57.91–160.87 nmol/L)	1.54					
	T_3	(1.23–3.07 nmol/L)	0.22					
	PRL	(2.7–26 $\mu\text{g/L}$)	0.15		0.38		0.50	
Clonidine stimulation	GH	($B' < 2.5 \mu\text{g/L}$)	0.065				0.01	0.045 0.03
Insulin-induced hypoglycemia								
	Insulin 0.1 U/kg							
	GH	($\mu\text{g/L}$)	0.01		0.01		0.015	
	Cortisol	(138–690 nmol/L)	751.55		731.96		749.90	
	Glucose	(3.9–6.1 mmol/L)	6.68		4.95		4.29	
	Insulin 0.15 U/kg							
GnRH stimulation	GH	($\mu\text{g/L}$)	0.165		0.065		0.02	
	Cortisol	(nmol/L)	866.33		632.36		713.48	
	Glucose	(mmol/L)	6.5		4.5		3.17	
	LH	(1.8–13.4 IU/L)	5.12	9.07		12.05	13.60	
	FSH	(3.0–12.0 IU/L)	9.39	10.44		9.98	11.94	



FIG. 1. The patient at 37 years of age. Large ears and a small plethoric face, with frontal bossing, and a retracted nasal bridge can be noticed.

chomotor development proceeded normally. Learning capacity was good. All 11 siblings have normal growth and development.

At 11 years and 7 months she was first seen by the Pediatric Endocrinology Unit. Height was 88 cm (44 cm below the third percentile) and weight was 15.5 kg.

At 16 years of age, she complained of abundant and irregular menses and constipation. Menarche occurred when she was 13 years old. Height was 111.5 cm and weight was 32.5 kg. She had clinical features of total GH deficiency (marked frontal bossing and a retracted nasal bridge) and hypothyroidism (infiltrated and dry skin, dry hair). Investigation revealed central hypothyroidism and levothyroxine (LT₄) therapy was started. Follow-up and treatment were irregular.

At 33 years of age, after a long period without follow-up, she complained of weight gain, dry skin, coldness, memory deficits, and irregular periods. She had discontinued LT₄ intake for 2 years. Height was 119 cm, weight was 47.5 kg and body mass index (BMI) was 33 kg/m². She was extremely hypothyroid; total T₄ was 3.86 nmol/L (normal, 58.0 to 161.0 nmol/L), total T₃ was 0.74 nmol/L (normal, 1.23 to 3.07 nmol/L) and TSH was 0.36 mU/L (normal, 0.43 to 3.8 mU/L). LT₄ therapy was reinitiated.

At 35 years of age, a low GH level was observed, which did not rise after a provocative test with clonidine (Table 1). At 38 years of age, PRL was measured for the first time, with low levels of 0.21 and 0.42 μ g/L on two separate occasions. LT₄ therapy was interrupted and she was admitted for investigation. She had many complaints and noticeable depression. Height was 119 cm, weight was 52 kg



FIG. 2. Pituitary MRI revealing anterior pituitary hypoplasia, as indicated by the arrowhead.

bryonic day 12 in the developing pituitary and disappears around the time of birth. The second population arises after the initial expression of Pit-1, and these thyrotrophs persist in adulthood (24). Whether such thyrotroph cell subpopulations also exist in humans is currently unknown. The observation of patients bearing *Pit-1* gene mutations with delayed appearance of TSH deficiency is in agreement with the hypothesis of the presence of two distinct thyrotroph populations in humans. In contrast to what has been shown in rodents, the putative Pit-1-independent thyrotroph cell population could indeed remain functional several years after birth, disappearing with advancing age (21). The patient presented in this report was investigated when she was already 38 years old and had a hypoplastic pituitary gland and absence of TSH response to TRH, both findings compatible with her age and the duration of Pit-1 deficiency.

The *Pit-1* mutation is sporadic in the majority of the cases, occurring in one allele of the gene. Mutant Pit-1 bearing the R271W mutation binds normally to DNA but there is decreased transactivation of the GH or PRL promoters, consistent with the clinical findings of GH and PRL deficiencies. The mutant protein works as a dominant inhibitor of transcription, forming dimers with the wild-type protein that results in complexes that do not activate transcription (15,18). TSH β gene, however, has a lower affinity for Pit-1, and this transcription factor appears to be less important for cell-specific expression of this gene in the anterior pituitary. The major functions of Pit-1 in the thyrotroph are to mediate hormonal responses to TRH, acting through the protein kinase-C pathway and to other neuropeptides acting through the protein kinase-A pathway (6,7,25). The presence of normal basal levels of TSH, but abnormal TRH-stimulated TSH secretion in patients with the R271W mutation is clinical evidence for this regulatory function (18). In our patient, despite the fact that her parents were consanguineous, the mutation was monoallelic and her parents and siblings were of normal stature, suggesting a sporadic *de novo* mutation. Molecular studies of other members of the family are being performed.

Pit-1 gene mutations may represent a more frequent cause of anterior pituitary hormone deficiency than previously recognized. Often, PRL determination, which is a clue to the diagnosis, is not done, and thyroid function is variable, with deficiency manifesting only after GH replacement therapy or with advancing age. Therefore, in some children with complete GH deficiency, the diagnosis may have been overlooked (21). The R271W is the most frequent mutation found in these cases, and it has been described in at least five unrelated cases of CPHD, whereas the others are isolated descriptions or shared by the same kindred (autosomal recessive inheritance) (3). Molecular studies of *Pit-1* gene, concentrating at codon 271, a "hot spot" for mutations (18), probably will elucidate many cases of CPHD and apparent isolated GH deficiency.

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