Novel Nonsense Mutation (p.Y113X) in the Human Growth Hormone Receptor Gene in a Brazilian Patient with Laron Syndrome

clinical case report

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ABSTRACT

Background: To date, about sixty different mutations within *GH receptor* (*GHR*) gene have been described in patients with GH insensitivity syndrome (GHI). In this report, we described a novel *nonsense* mutation of GHR. **Methods:** The patient was evaluated at the age of 6 yr, for short stature associated to clinical phenotype of GHI. GH, IGF-1, and GHBP levels were determined. The PCR products from exons 2–10 were sequenced. **Results:** The patient had high GH (26 µg/L), low IGF-1 (22.5 ng/ml) and undetectable GHBP levels. The sequencing of *GHR* exon 5 disclosed adenine duplication at nucleotide 338 of *GHR* coding sequence (c.338dupA) in homozygous state. **Conclusion:** We described a novel mutation that causes a truncated GHR and a loss of receptor function due to the lack of amino acids comprising the transmembrane and intracellular regions of GHR protein, leading to GHI. **(Arq Bras Endocrinol Metab 2008; 52/8:1264-1271)**

Keywords: Growth hormone insensitivity; Somatotropin receptors; Genetics; Growth

RESUMO

Nova Mutação Nonsense (p.Y113X) no Gene do Receptor do Hormônio do Crescimento em um Paciente Brasileiro com Síndrome de Laron.

Introdução: Até o momento, aproximadamente 60 diferentes mutações envolvendo o gene do receptor do GH (GHR) foram descritas em pacientes com a síndrome de insensibilidade ao GH (GHI). Neste artigo, descrevemos uma nova mutação *nonsense* do GHR. **Métodos:** O paciente foi avaliado aos 6 anos de idade para baixa estatura associada ao fenótipo clínico da GHI. Níveis de GH, IGF-1 e GHBP foram determinados. Os produtos de PCR dos éxons 2-10 foram seqüenciados. **Resultados:** O paciente apresentou níveis elevados de GH (26 µg/L), baixos de IGF-1 (22.5 ng/ml) e indetectáveis de GHBP. O seqüenciamento do éxon 5 do GHR revelou uma duplicação da adenina no nucleotídeo 338 da sequência de codificação do GHR (c.338dupA) em homozigose. **Conclusão:** Descrevemos uma nova mutação que causa um GHR truncado e uma perda da função do receptor devido à perda de aminoácidos compreendendo as regiões transmembrana e intracelular do receptor, levando a GHI. **(Arq Bras Endocrinol Metab 2008; 52/8:1264-1271)**

Descritores: Insensibilidade ao hormônio do crescimento; Receptores do hormônio do crescimento; Genética; Crescimento

INTRODUCTION

The Laron syndrome (LS) is a genetic disorder defined as the inability to respond to endogenous or exogenous GH with appropriate growth and metabolic effects. It is caused by defects in the GH receptors (GHR). Clinically, growth hormone insensitivity (GHI) is characterized by severe growth failure after birth,

craniofacial disproportion, elevated serum GH and low IGF-I that failed to respond to GH (1-3).

LS is caused, in most cases, by a fully penetrant autosomal recessive mechanism leading to genetic defects along the GH-IGF axis, including the GHR. The GHR gene on the short arm of chromosome 5 includes 9 exons comprising 620 amino acid residues in its mature form. The GHR protein can be divided into the extracellular domain (encoded by exons 2–7), the transmembrane domain (encoded by exon 8), and the intracellular domain (encoded by exons 9 and 10) (4,5).

The first genetic defect of the GHR described was a deletion of exons 5 and 6 which encode a large extracellular GH-binding domain. Since then, about sixty mutations of the GHR gene have been described (6).

Ethnic origin of reported cases is predominantly Middle Eastern, Mediterranean, South Asian and Ecuadorian. In Brazil twelve patients with the LS have been reported so far, including six children from Orobó (Pernambuco state) carrier of the E180 splice site mutation (7). Another case, also from Pernambuco (city of Recife), was described in 1997 (8) and, in this paper, a novel mutation in the exon 5 of GHR in this patient is described for the first time.

SUBJECTS AND METHODS

Case report

The patient was evaluated for short stature, soon after adoption. Birth length was 44 cm and weight 3 kg. At the age of 6.5 years, his height was 77 cm [height standard deviation score (SDS) –8.0], weigh 9 kg [90% of ideal body weight for height (IBW)] and bone age of 3 years.

When he was 10 yr 9 mo of age, he was reevaluated and measured 86 cm in height (height SDS –8.8) and weighed 12 kg (100% IBW). At 12 years of age his height was 87.8 cm (height SDS -8.4) and his weight 11.1 kg (89% IBW). Head circumference was 49.1cm, 25th percentile for his height (ie normal head circumference for 24 months).

He had facial asymmetry, prominent forehead, depressed nasal bridge, short vertical dimension of the face, microstomia and blue sclerae. There was severe dental crowding and caries of retained primary dentition. His voice was high pitched. There was limited elbow extension of approximately 5 degrees bilaterally. Stretched penile length was 4 cm (10th percentile for age 3) and the testes were 1mL bilaterally.

After the diagnosis of GHI, he was treated with human recombinant IGF-I (Genentech, South San Francisco, CA) at a dosage of 100µg/kg/body weight twice daily by subcutaneous injection. During the subsequent 6 months he grew 3.1 cm at the rate of 6 cm/year.

Hormonal studies

The GH was determined by quimioluminescence (Immulite-DPC 2000). The assay was calibrated against WHO IRP 80/505 and recognized 22 kDa GH isoform; interassay coefficient of variation was 4,6%. The IGF-1 and IGFBP-3 were measured using immunoradiometric assay (Immulite-DPC 2000) and interassay coefficient of variation were 3,9 e 4,8%, respectively. The GHBP was determined by ligand-mediated immunofunctional assay (LIFA; Esoterix, Austin, TX, USA).

IGF-1 generation test involved daily injections of recombinant human GH (0,1 U/kg subcutaneously) for 4 days. Blood samples were drawn in the morning before the first injection and 12 hours after the last dose. IGF-1 increase < 15 µg/L was considered for the diagnosis of GHI.

Molecular studies

Genomic DNA was isolated from peripheral blood leucocytes from the patient and 50 normal height controls. We studied the GHR gene of the patient, amplifying exons 2–9 using specific intronic primers and exon 10 in three overlapping fragments to cover the entire coding region.

PCR was carried out in a 25-µl mixed solution, containing 50-200 ng genomic DNA, 20 pmol/L each pair of primers, 0.2 mmol/L each of deoxy-ATP, -GTP, -CTP, -TTP, 1.5 to 4.0 mmol $MgCL_2$, 0.5 U Taq DNA polymerase, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.3) (Table 1). PCR involved initial denaturation step for 5 min at 95 °C, 35 cycles of denaturation for 1 min at 94 °C, primer annealing for 1 min at 55 C, and primer extension for 1 min at 72 C, followed by a final extension step for 10 min at 72 C in a thermal-cycler. PCR products were viewed after electrophoresis on 2% (wt/vol) agarose gel stained with ethidium bromide and photographed under UV light. PCR products from exons 2–10 were directly sequenced with dideoxy chain-termination method using a kit ABI PrismTM BigDye Terminator (Perkin Elmer, Foster City, CA, USA) and analysed by an autosequencer ABI Prism Genetic Analyser 3100 automatic DNA sequencer (Perkin Elmer). Exon 5 of 50 control samples were amplified by PCR and directly sequenced.

Table 1. Primer pairs to amplify the GHR coding sequence, sizes of PCR products and final MgCl2 concentration for PCR reaction.

Exon	Primer	PCR product (bp)	($MgCl_2$)
2	**Sense:** 5′ TTTCATGATAATGGTCTGCT 3′	193	1.5 mM
	Antisense: 5′ GAATACAGTTCAGTGTTGTTT 3′		
3	**Sense:** 5′ GATGGACTAGATGGTTTTGCCTTCCTCTTTCTGTTTCAG 3′	100	1.5 mM
	Antisense: 5′ GGATAGTAGCTTAATTACAC 3′		
4	**Sense:** 5′ AGGATCACATATGACTCACC 3′	231	2.5 mM
	Antisense: 5′ AGGAAAATCAGAAAGGCATG 3′		
5	**Sense:** 5′ ACTTAAGCTACAACATGATT 3′	274	4.0 mM
	Antisense: 5′ GCTTCCCCATTTATTTAGT 3′		
6	**Sense:** 5′ ATTGTGTCTGTCTGTGTACTAATG 3′	247	1.5 mM
	Antisense: 5′ ATAGAAAGAAAAGTCAAAGTGTAAG 3′		
7	**Sense:** 5′ TAGTGTTCATTGGCATTGAG 3′	328	3.0 mM
	Antisense: 5′ ACAAAAGCCAGGTTAGCTAC 3′		
8	**Sense:** 5′ GAAACTGTGCTTCAACTAGTC 3′	205	4.0 mM
	Antisense: 5′ GGTCTAACACAACTGGTACA 3′		
9	**Sense:** 5′ GCTATAATTGAGAATATGTAG 3′	204	1.5 mM
	Antisense: 5′ CATATGACAGGAGTCTTCAGGTG 3′		
10	**Sense:** 5′ GAGTTTCTTTTCATAGATCTTCATTTTC 3′	1.150	1.5 mM
	Antisense: 5′ GGTTTAAACATTGTTTTGGC 3′		
	A*: 5′ GTTGCTCAGCCACAGAGGTT 3′		
	B*: 5′ CCAGCAGGTAGTGTGGTCCT 3′		

* primers used only for sequence.

RESULTS

Hormonal results

During insulin-induced hypoglycemia stimulation test, GH rose from a high baseline of 26 µg/L to 52 µg/L at 45 minutes. IGF-I level was low at 22.5 ng/ml (normal for age: 80-650 ng/ml) and the IGFBP-3 level was also low at 100 ng/ml (normal for age: 2000-6000 ng/ml). After exogenous GH injection (0.1U/kg/day) for 4 days, the serum IGF-I was 24ng/dl.

At the age of 12 years, he was evaluated at the University of North Carolina to entry into a treatment program with recombinant IGF-I injections and was found to have an IGF-I level in serum of <10 ng/ml, and a GH level of 22 µg/L. After 4 days of GH injections (0.1 U/kg/day), there was no change in IGF-I serum concentration. The serum concentration of GH-binding protein (GHBP) was undetectable (<140 pmol/l; normal for ages 10-15 yr, 431-1892 pmol/l).

Molecular results

Direct sequence of *GHR* exon 5 (DNA reference sequence: NM_000163) disclosed an adenine duplication at nucleotide 338 of the *GHR* coding sequence (c.338dupA) and was observed in homozygous state in the patient`s genomic DNA. This allelic variant was not found in 100 alleles from the control group and causes a *nonsense* mutation through the conversion of tyrosine (TAC) into a premature termination signal (TAA) (p. Y113X). This stop codon occurs in the extracellular domain and produces a truncated GHR that would be unable to exhibit receptor function, due to the lack of 525 amino acids comprising the transmembrane and intracellular regions of GHR protein.

DISCUSSION

Growth hormone insensitivity (GHI) is a rare disorder and 12 Brazilian patients have been reported to date (7), including the present patient (8). All these patients presented with the typical clinical features of LS with elevated serum GH, low IGF-I which failed to respond to GH, and low GHBP.

Among Brazilian GHI patients, molecular studies were conducted in 6 cases, and all of them presented homozygous mutations in *GHR* gene. The first two GHI cases reported from Brazil were homozygous for a substitution of T for G at the -1 position of the 3' splice consensus sequence of intron 6 (c.619-1 G>C) (9, 10). Another patient carried a homozygous mutation, replacing serine by isoleucine in codon 244 of exon 7 (p.S244I) (11). The last six patients described in Brazil were also from Pernambuco and carried a homozygous replacement of guanine by adenine in codon 198 of exon 6 (c.594 A>G), creating an abnormal splice site deleting 8 amino acids from the extracellular domain of GHR (p.V199_M206del) (7). To date, this is the most common mutation present in Brazil, as well as in the world. The mutation in codon 198, also known as E180Splice mutation, has been described in Ecuadorian patients and two Israeli patients of Moroccan descent (12,13).

Finally, in the present study, we describe a novel *nonsense GHR* mutation in homozygous state, adenine duplication at nucleotide 338 (c.338dupA). This nucleotide alteration causes a premature termination signal at condon 113 (p.Y113X), predicting a truncated GHR that would be unable to exhibit receptor function due to the lack of amino acids comprising the transmembrane and intracellular regions of GHR protein. This molecular finding is in accord with undetectable levels of GHBP observed in our patient. GHBP is a product of proteolytic cleavage of the extracellular domain of GHR and is typically absent or present at low levels in patients with GHI, reflecting failure of GH receptor synthesis (14).

To date, approximately 60 *GHR* defects have been described, such as gross deletions, missense, *nonsense*, frameshift, and splice site mutations. Fourteen *nonsense* mutations in *GHR* were described (Table 2), being 2 of them located in exon 5 (22,23).

Table 2A. Standard and colloquial nomenclature for *GHR* mutations (Gross Deletion and Nonsense mutations).

Type of Mutation	DNA sequence change*	Site of mutation	Amino acid change	Colloquial nomenclature	Reference
Deletion					
	~ 1.2 kb deletion	Partial Exon 5 deletion			(15)
	~ 4 kb deletion	Exon 5			(16)
	~ 19 kb deletion	Exon 5-6			(17)
	del(5) p11-p13.1	Exon 4-10			(18)
Nonsense					
	c.12 G>A	Exon 2	p.W4X	W15X	(19)
	c.101 G>A	Exon 3	p.W34X	W16X	(20)
	c.168 C>A	Exon 4	p.C56X	C38X	(21)
	c.181 C>T	Exon 4	p.R61X	R43X	(21)
	c.247 C>T	Exon 4	p.Q83X	Q65X	(22)
	c.293 G>A	Exon 5	p.W98X	W80X	(22)
	c.303 C>A	Exon 5	p.C101X	C83X	(23)
	c.338 dupA	Exon 5	p.Y113X	Y97X	#
	c.476 T>A	Exon 6	p.L159X	L141X	(19)
	c.524 G>A	Exon 6	p.W175X	W157X	(22)
	c.601 G>T	Exon 6	p.E201X	E183X	(13)
	c.703 C>T	Exon 7	p.R235X	R217X	(24)
	c.724 G>T	Exon 7	p.E242X	E224X	(25)
	c.744 delT	Exon 7	p.Y248X	230delT	(26)

present study

Table 2B. Standard and colloquial nomenclature for *GHR* mutations (Frameshift and splice site mutations).

Frameshift					
	c.162delC	Exon 4	p.K55fsX79	162delC	(22)
	c.192_193delTT	Exon 4	p.S65fsX70	46delTT	(9)
	c.421_422dupTT	Exon 5	p.L143fs147	422insTT	(6)
	c.743_744delAT	Exon 7	p.Y248fs259	230 del AT	(9)
	c.889_911del	Exon 9	p.I297fs299	889-911del	(27)
	c.981delC	Exon 10	p.I328fs348	981delC	(25)
	c.1324_1345del	Exon 10	p.A442fs478	GHR(1-499)	(28)
	c.1734delG	Exon 10	p.R578fs600	1776 del G	(23)
Splice					
	c.594 A>G	Exon 6	p.V199_M206del	E180Splice	(29)
	c.723 C>T	Exon 7		G236Splice	(22,30)
	c.875 G>C	Exon 8	p.R292T	R274T	(31)
	c.70+1 G>A	Intron 2		70+1 G>A	(22)
	c.266+1 G>A	Intron 4		71+1 G>A	(9)
	c.440-1 G>C	Intron 5		IVS5 -1 G>C	(24)
	c.618+1.8kb A>G	Intron 6		ψ6	(32)
	c.619-1 G>T	Intron 6		189-1G>T	(9)
	c.619-1 G>C	Intron 6		189-1G>C	(33)
	c.619-25 A>G	Intron 6		IVS 6 -25 A>G	(29)
	c.785-1 G>T	Intron 7		785-1 G>T	(34)
	c.876-1 C>G	Intron 8		GHR(1-277)	(35)
	c.945+1 G>A	Intron 9		GHR(1-277)	(36)

The description of *GHR* mutations started several years before any mutation nomenclature recommendations were proposed. Therefore, published and commonly used designations for many *GHR* mutations have been at variance with the evolving standard nomenclature guidelines (43). In Table 1, we reviewed all *GHR* mutations and disclosed the standard nomenclature and colloquial nomenclature side by side. Nucleotide numbering is based on cDNA reference sequence (NM_000163) and amino acid numbering is based on GHR protein reference (NP_000154), being the translation initiator Methionine numbered as +1 codon, in contrast to the colloquial notations that numbered the ATG initiation codon as -18.

GHR mutations are concentrated in extracellular domain, mainly in exons 4, 5, 6 and 7; whereas mutations in the exons 8, 9 and 10 are rare (Table 1). Although there are rare cases of autosomal dominant inheritance, the majority of cases of GHI are caused by an autosomal recessive mechanism or exhibit compound heterozygous defects. Most cases are associated with parental consanguinity (4,14,44), but such data are not available for this patient.

Table 2C. Standard and colloquial nomenclature for *GHR* mutations (Missense mutation and others).

Missense					
	c.1 A>T	Exon 2	p.0?	M-18L	(37)
	c.166 T>A	Exon 4	p.C56S	C38S	(22)
	c.173 C>T	Exon 4	p.S58L	S40L	(22)
	c.148 G>A	Exon 4	p.E60K	E42K	(38)
	c.202 T>C	Exon 4	p.W68R	W50R	(22)
	c.266 G>A	Exon 4	p.R89K	R71K	(24)
	c.310 T>G	Exon 5	p.Y104D	Y86D	(26)
	c.335 G>C	Exon 5	p.C112S	C94S	(4)
	c.341 T>C	Exon 5	p.F114S	F96S	(39)
	c. 428 T>C	Exon 5	p.V143A	V125A	(24)
	c.446 C>A	Exon 6	p.P149Q	P131Q	(40)
	c.485 T>A	Exon 6	p.V162D	V144D	(24)
	c.504 T>G	Exon 6	p.H168Q	H150Q	(4)
	c.508 G>C	Exon 6	p.D170H	D152H	(41)
	c.512 T>C	Exon 6	p.I171T	I153T	(27)
	c. 515 A>C	Exon 6	p.Q172P	Q154P	(27)
	c. 518 T>G	Exon 6	p.V173G	V155G	(27)
	c.535 C>T	Exon 6	p.R179C	R161C	(24)
	c.685 C>G	Exon 7	p.R229G	R211G	(24)
	c.731 G>T	Exon 7	p.S244I	S226I	(11)
	c.677 A>G	Exon 7	p.Y226C	Y208C	(42)
	c.784 G>A	Exon 7	p.D262N	D244N	(42)

* Nucleotide numbering is based on DNA reference sequence NM_000163. Number nucleotides and name mutations or variants, respectively, according to the standard nomenclature recommendations (http://www.HGVS.org/mutnomen/).

Rarely, GHI may also result from mutations in signal transducer and activator of transcription 5B gene (*STAT5b;* OMIM: 604260), the main component of the GH signaling pathway (5). STAT5b also plays an important role in signaling within immune cells, thus clinical immunodeficiency is common in patients with STAT5b deficiency, although is not an obligatory phenomenon (4,45,46).

In conclusion, we expand the repertoire of GHR mutations describing the novel mutation (c.338dupA; p.Y113X) in a Brazilian GHI patient.

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