

# Influence of growth hormone receptor (GHR) exon 3 and -202A/C IGFBP-3 genetic polymorphisms on clinical and biochemical features and therapeutic outcome of patients with acromegaly

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## Abstract

**Background** The association of GHR-exon 3 and -202 A/C IGFBP3 polymorphisms with clinical presentation, biochemical measurements and response to therapies in acromegaly have been suggested.

**Objective** To evaluate the presence of these polymorphisms in acromegaly and their influence on clinical and laboratorial characteristics of patients at diagnosis and after treatment in a large cohort of acromegalic patients.

**Patients and methods** This is a cross-sectional study developed in a single tertiary reference center. Clinical data were obtained from the medical records of 186 acromegalic patients (116 women, age range 21–88 years). GH and IGF1 levels and GHR-exon 3 and -202 A/C IGFBP3 polymorphisms were evaluated in the same hospital.

**Results** At diagnosis, serum GH concentrations were lower in patients with GHR-d3 genotype than those with GHR-fl, whereas an association of lower IGFBP3 levels with d3 allele was observed only after neurosurgical or medical treatments. However, these associations were not confirmed in posterior statistical analysis.

**Conclusion** Our results suggest that GHR-exon 3 and -202 A/C IGFBP3 polymorphisms did not show any consistent association on clinical and laboratorial features of acromegalic patients even after treatment.

**Keywords** Acromegaly · Pituitary tumors · GHR-exon 3 genotype · -202 A/C IGFBP3 polymorphism

## Introduction

Acromegaly is a rare disorder caused by growth hormone (GH) hypersecretion which, through binding to the growth hormone receptor (GHR), leads to increased production of insulin-like growth factor 1 (IGF-1). In most, but not in all patients, the clinical phenotype correlates with the degree of GH/IGF-1 hypersecretion and the relationship between GH and IGF1 levels is linear in active disease [1, 2]. Ideally, serum GH and IGF-1 values should be concordant at diagnosis and in the assessment of disease activity during treatment. Nevertheless, discrepancy between GH and IGF-1 levels (normal GH and elevated IGF1 or vice versa) has been reported [3–8].

Common genetic variants within GH-IGF-1 axis may cause differences in the GH/GHR signaling pathways and were described as an important contributor to height variation in the general population and to rhGH therapy response in growth retarding disorders [9]. GHR-exon 3 polymorphism in the GHR gene is one of these variations. This polymorphism gives rise to two isoforms of GHR transcripts differing by the retention (GHR-fl) or exclusion (GHR-d3) of exon 3 [10, 11]. The effect of GHR-exon 3 polymorphism is still unclear, as it does not affect the affinity, binding capacity, or internalization of d3 and fl GHR isoforms. However, it could facilitate ligand-induced activation of GHR [10, 12–15].

In clinical practice, the presence of the GHR-d3 genotype has been associated with enhanced sensitivity to exogenous recombinant human GH administration in children with Turner syndrome, in those with short stature

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small-for-gestational-age, and in those with severe GH deficiency [2, 5]. In acromegaly, patients harboring the GHR-d3 allele have lower levels of circulating GH compared with the GHR-fl homozygous carriers [16]. Moreover, the presence of GHR-d3 isoform was associated with more severe clinical picture and a lower chance of biochemical control [17].

The polymorphism -202 A/C (rs2854744) in the promoter region of the Insulin-like growth factor binding protein-3 (IGFBP3) gene, which encodes a protein that regulates IGF-1 action, has also been involved in GH therapy pharmacogenetics of GH deficiency and in children born small for gestational age [18, 19]. The -202 A allele was associated with a significantly decreased circulating level of IGFBP-3 in both in vitro and in vivo studies [20, 21]. However, only one study evaluated the -202 A/C IGFBP3 in acromegaly showing no significant difference in clinical characteristics according this genotype.

This study aimed to evaluate the possible influences of GHR-d3 and -202 A/C IGFBP3 polymorphisms on the clinical and metabolic presentation of a large population of Brazilian acromegalic patients, at diagnosis and after treatment, and to investigate the impact of these polymorphisms on the prevalence of long-term complications of acromegaly.

## Materials and methods

### Patient characteristics

A cohort of 186 acromegalic patients (116 females) followed at the Neuroendocrinology Unit of the Clinical Hospital of the University of São Paulo Medical School were evaluated. Mean age was 43.68 ( $\pm$ 13.63) years, ranging from 21–88 years. The interval from the presumed onset of symptoms until diagnosis was  $8 \pm 6$  years; mean body mass index (BMI) was  $31.77 (\pm 18.8)$  kg/m<sup>2</sup>. The clinical suspicion of acromegaly was confirmed by serum IGF-I level above the upper limit of normal range and by lack of GH suppression  $<1.0$  ng/mL during an oral glucose load. At diagnosis, GH and IGF-1 levels were  $3.46 \pm 16.31$  and  $937.77 \pm 438.30$ , respectively; and the baseline % ULNR-IGF-1 was  $334 \pm 160$ . All patients had a magnetic resonance imaging (MRI) of the sella region, and 97 % ( $n = 181$ ) harbored macroadenomas (10 mm in diameter). Four macroadenomas showed radiological evidence of tumor necrosis or hemorrhage, suggesting apoplexy. Data was expressed as mean  $\pm$  SD.

At the time of diagnosis, two or more comorbidities were presented in acromegalic patients: hypertension (74 %), type 2 diabetes (38 %), glucose intolerance (35 %), arthropathy (73 %); cardiovascular disease (38 %), carpal tunnel

syndrome (5 %); colon polyps (36 %); goiter (61 %) and papillary thyroid cancer (3.7 %). Two patients had colorectal cancer, 50 hyperplastic polyps, 3 adenomatous/hyperplastic polyps, 20 with adenomatous polyps and 101 had normal colonoscopy (Table 1).

The primary treatment was transsphenoidal surgery (TSS) in 120 patients, somatostatin analogs in 52, dopamine agonists in 13 patients (2 on bromocriptine and 11 on cabergoline) and radiotherapy in one patient. In those patients submitted to TSS, the GH-secreting nature of their tumors was confirmed by positive immuno-histochemical staining for GH. Clinical, biochemical and imaging assessment were performed 3–6 months postoperatively. For those that did not obtain control of the disease, adjuvant treatment with surgery, radiotherapy and/or medication was introduced (Table 2).

Local ethics committee approved this study and all patients provided written informed consent.

### Hormone assays

Serum GH concentration was measured before 1994 with immunoradiometric assay (IRMA) in eleven patients; subsequently (1994 onwards), GH samples of the others patients were measured by immunofluorometric assay (IFMA) (AutoDELFIA, Wallac, Turku, Finland) with monoclonal antibodies. IGF-1 was measured by RIA after ethanol extraction (Diagnostic Systems Laboratories, Webster, TX) or by chemiluminescence assays (CLIA) (IMMULITE; Diagnostic Products Corp., Los Angeles, CA). IGFBP3 was measured by IRMA (Diagnostic Systems Laboratories) or CLIA (IMMULITE). IGF-1 and IGFBP3 absolute levels were standardized for age and sex, according to reference values provided by the respective assay kits. To achieve a standardized comparability value among the two different assay used along these years, IGF-I levels were also expressed as the

**Table 1** Prevalence of comorbidities in acromegalic patients ( $n = 186$ ) at diagnosis

Comorbidities	Patients (%) <sup>*</sup>
Hypertension	75
Diabetes mellitus	38
Glucose intolerance	35
Arthropathy	73
Cardiovascular disease	38
Carpal tunnel syndrome	5
Colon polyps	36
Goiter	61
Papillary thyroid cancer	3.7

<sup>\*</sup> Some patients had more than one comorbidity. Therefore, total is  $>100$  %

**Table 2** Treatments received by acromegalic patients ( $n = 186$ )

Treatments	<i>N</i> (%)
Primary	
Surgery	120 (64.5)
Radiotherapy	1 (0.5)
Somatostatin analogs	52 (28)
Dopamine agonists	13 (7)
Throughout time	
Surgery	38 (20.4)
DA	4 (2.2)
Surgery + SA	12 (6.5)
Surgery + RT	15 (8.1)
DA + SA	24 (12.9)
DA + Surgery	3 (1.6)
DA + Surgery + SA	74 (39.8)
DA + SA + RT	1 (0.5)
DA + Surgery + RT	3 (1.6)
DA + Surgery + SA + RT	12 (6.5)

RT radiotherapy, SA somatostatin analogs, DA dopamine agonists

percentage above the upper limit of the normal range (% ULNR-IGF-1).

#### Definition of disease control/disease remission

Normal serum IGF-I levels adjusted for age and random GH levels below 1.0 ng/mL defined biochemical control during medical treatment. In patients submitted to surgery, disease remission was defined by normal IGF-1 with GH nadir below 1.0 ng/mL during oral 75-g glucose load (OGTT), performed approximately 3–4 months after the procedure. Controlled patients after surgery and/or radiotherapy or during drug treatment were collectively defined as being “in remission” of the disease. The number of previous treatments until the visit study was recorded. This number was used as a potential predictive factor of aggressive phenotype, characterized by persistence of disease after surgical, medical therapy and radiotherapy, reflecting a poor response to usual treatment.

#### GHR-exon 3 and -202 A/C IGFBP3 genotyping

Genomic DNA was obtained from peripheral blood leukocytes using standard methods from all patients. DNA was amplified by multiplex PCR to determine the GHR-exon 3 polymorphism using previously described primers and PCR condition (16). The -202 A/C IGFBP3 (rs2854744) polymorphism was determined by a specific real-time PCR-based genotyping assay (ID 186389191-1, TaqMan SNP genotyping assay; Applied Biosystems) according to the manufacturer’s instructions in a StepOnePlus™ Real Time PCR

system (Applied Biosystems, Foster City, CA). Fifteen percent of all samples were randomly genotyped by direct sequencing for checking of assay performance and quality control. The agreement of the genotypes determined in both analyses was 100 %.

#### Statistical analysis

Qualitative variables are expressed as frequencies and percentages, whereas quantitative data are presented as mean  $\pm$  SD. The distribution of genotypes was determined and then compared with clinical and hormonal characteristics at diagnosis and after treatment. ANOVA followed by a Tukey test was used for comparisons according to the additive model, whereas the *t* test was used for comparisons according to the dominant model. Numerical variables that did not demonstrate parametric distribution were analyzed by Kruskal–Wallis one-way ANOVA on ranks or Mann–Whitney rank sum test. Nominal variables were compared by a  $\chi^2$  or Fisher exact test, as appropriate. Serum GH levels were log<sub>10</sub> transformed in order to normalize their distributions. To assess whether genetic variants had an independent effect, we performed single-followed by multiple-regression analyses adjusting for the established influential factors. SigmaStat for Windows (version 3.5; SPSS, Inc., San Rafael, CA) performed all statistical analyses. *P* value <0.05 was considered statistically significant.

#### Results

Eighty-three (45 %) patients were to be homozygous for GHR-fl, 81 (44 %) heterozygous and 22 (12 %) homozygous carriers of the GHR-d3. At diagnosis, serum GH concentrations were lower in patients with GHR-d3 allele ( $P = 0.026$  and  $P = 0.007$ , according additive and dominant model, respectively; Table 3). After treatment, this association did not persist and a lower IGFBP3 levels was observed in patients carrying the GHR-d3 genotype ( $P = 0.009$  and  $P = 0.033$ , according additive and dominant model, respectively; Table 3). However, multiple linear regression analysis did not confirm these associations ( $R = 0.3/P = 0.057$  and  $R = 0.7/P = 0.075$ , for GHR-d3 vs. serum GH and IGFBP3, respectively) but showed, as expected, a strong correlation between log<sub>10</sub> GH and ULNR of IGF-1 ( $R = 0.3/P = < 0.001$ ) and log<sub>10</sub> GH and age ( $R = 0.3/P = 0.005$ ).

In relation to IGFBP3, polymorphism -202A/C was detected in homozygosis in 38 (20 %) and heterozygosis in 95 (51 %), whereas 53 (28 %) of patients were homozygous for wild type C allele. After treatment, a lower basal serum GH concentration was observed in patients carrying allele A

**Table 3** Comparisons of clinical and laboratory characteristics of acromegalic patients in accordance with GHR-exon 3 genotypes at diagnosis and after treatment

GHR-exon 3	fl/fl	fl/d3	d3/d3	d3/d3 and fl/d3	$P^a$	$P^b$
Sex (F/M)	54/29	50/31	12/10	62/41	0.656	0.496
Age (years)	46.9 ± 13.6	52.2 ± 13.2	43.9 ± 13.8	50.4 ± 13.7	0.009	0.084
BMI (kg/m <sup>2</sup> )	29,1 (26,5–32,9)	28,9 (26,4–32,7)	30,2 (25,7–33,9)	29,0 (26,3–32,7)	0.964	0.903
DM (yes/total)	30/53	29/52	11/11	40/63	0.443	0.707
Tumor (micro/macro)	2/81	3/78	0/22	3/100	0.621	0.833
<i>At diagnosis</i>						
bGH <sup>c</sup> (ng/mL)	26.3 (9.5–47.8)	12.9 (7.0–29.3)	14.3 (3.6–42.3)	12.9 (6.8–29.3)	0.026	0.007
nGH	14.1 (5.6–32.1)	7.9 (3.7–22.7)	6.9 (2.1–24)	7.9 (3.2–23.5)	0.208	0.077
IGF-1 (ng/mL)	922 (637–1,275)	893 (649–1,159)	722 (477–906)	832 (602–1,105)	0.069	0.211
ULNR-IGF-1(%)	283 (200–456)	315 (237–438)	222 (187–298)	300 (205–426)	0.019	0.940
IGFBP3 (mg/L)	7.6 (6.5–8.5)	7.3 (6.3–8.1)	7.1 (6.2–8.3)	7.3 (6.3–8.2)	0.379	0.165
<i>After treatment</i>						
Remission (yes/total)	22/56	22/58	9/12	31/70	0.366	0.718
bGH (ng/mL)	1.0 (0.4–3.2)	0.9 (0.4–1.7)	0.8 (0.2–2.0)	0.9 (0.4–1.8)	0.222	0.114
nGH	0.25 (0.1–0.7)	0.5 (0.2–2.25)	0.3 (0.1–0.65)	0.5 (0.12–1.77)	0.420	0.387
IGF-1 (ng/mL)	294 (175–529)	255 (178–387)	223 (199–382)	248 (180–384)	0.565	0.291
ULNR-IGF-1 (%)	119 (70.4–189)	110 (81.5–161)	96.9 (77.6–127)	106 (80.4–159)	0.625	0.497
IGFBP3 (mg/L)	5.3 (4.2–6.4)	4.9 (4.1–5.9)	4.3 (3.9–4.8)	4.7 (4.1–5.5)	0.009	0.033

Values are expressed as mean ± SD (range)

DM diabetes mellitus, bGH baseline GH, nGH nadir GH, ULNR upper limit of the normal age- and sex-matched range

<sup>a</sup> Additive (or co-dominant) model: fl/fl × fl/d3 × d3/d3

<sup>b</sup> Dominant model: fl/fl × (fl/d3 + d3/d3)

<sup>c</sup> Serum GH data are log<sub>10</sub> transformed

( $P = 0.040$  and  $P = 0.018$ , according additive and dominant model, respectively (Table 4).

The proportion of patients having comorbidities and requiring multiple forms of therapy (surgery, radiotherapy and pharmacological therapy) were similar between three genotypes for both GHR-exon3 and -202 A/C IGFBP3 variants (data not shown).

All polymorphisms evaluated followed Hardy–Weinberg equilibrium.

## Discussion

The association of the GHR-exon 3 and -202 A/C polymorphisms on the clinical features, comorbidities and responses to medical treatment were evaluated in a large cohort of acromegalic patients ( $n = 186$ ). The genotype frequency of GHR-exon 3 polymorphism found in the present study are similar to that observed in healthy subjects [10, 12] and in other reports of acromegalic patients [16, 17, 22–29] where, homozygous for GHR-fl is the predominant genotype and almost half of the individuals tested are carriers of at least one d3-GHR allele (Table 5).

The presence of d3 allele was found to be associated with a tendency of lower GH concentrations at diagnosis ( $P = 0.007$ ) and lower IGFBP3 levels after treatment ( $P = 0.033$ ). However, when we used multiple linear regression to combine log<sub>10</sub> GH and IGFBP3 concentrations, % ULNR-IGF-1, age and GHR-exon 3 genotypes, these associations does not persist but display almost significant tendency ( $P = 0.057$  and  $0.075$ , respectively). In this analysis, the only variable that affected basal GH was age ( $P = 0.005$ ) whereas % ULNR-IGF-1 was influenced by log<sub>10</sub> GH ( $P < 0.001$ ).

Schmid et al. [16] first demonstrated the influence of GHR-exon 3 polymorphism on endogenous GH concentrations, evaluating untreated acromegalic patients at diagnosis. These authors reported that basal GH was significantly higher in the GHR-fl than in the GHR-d3 group, whereas IGF1 concentrations were comparable in both groups; thus, it was suggested that lower GH concentrations are required for patients carrying d3 allele to produce a given increase in serum IGF-1 concentrations and to develop acromegalic symptoms due to higher sensitivity of GHR-d3 isoform [16]. GHR-d3 showed an increased rate of GH and IGF-I levels (high IGF-I levels and GH ≤2 ng/ml) after somatostatin analogs treatment.

**Table 4** - Comparisons of clinical and laboratory characteristics of acromegalic patients in accordance with the -202 A/C IGFBP3 polymorphism at diagnosis and after treatment

-202 A/C IGFBP3	C/C	A/C	A/A	A/C and A/A	$P^a$	$P^b$
Sex (F/M)	31/22	60/35	24/4	84/49	0.806	0.514
Age (years)	46.9 ± 13.0	48.9 ± 13.3	50.8 ± 15.6	49.5 ± 14.0	0.404	0.254
BMI (kg/m <sup>2</sup> )	28.5 (26.2–32.8)	29.4 (26.5–32.8)	28.9 (26.2–32.8)	29.2 (26.3–32.8)	0.927	0.919
DM (yes/total)	25/28	32/63	13/25	45/88	0.253	0.097
Tumor (micro/macro)	1/52	3/92	1/37	4/128	0.896	0.665
<i>At diagnosis</i>						
bGH <sup>c</sup>	18.9 (8.1–41.8)	15.0 (6.6–35.0)	22.0 (8.3–58.2)	15.7 (6.7–40.0)	0.298	0.353
nGH	9.3 (4.4–27.4)	8.9 (4.7–25.6)	16.0 (3.7–27.6)	9.5 (3.6–26.0)	0.736	0.564
IGF-1 (ng/mL)	906 (648–1,140)	789 (563–1,159)	937 (744–1,323)	869 (595–1,182)	0.305	0.582
ULNR-IGF-1 (%)	297 (207–438)	278 (200–412)	374 (225–506)	294 (202–433)	0.228	0.801
IGFBP3 (mg/L)	7.3 (6.2–8.3)	7.3 (6.3–8.3)	7.6 (6.8–8.4)	7.4 (6.4–8.3)	0.512	0.595
<i>After treatment</i>						
Remission (yes/total)	10/42	32/59	10/25	42:84	0.131	0.060
bGH (ng/mL)	1.3 (0.7–3.95)	0.65 (0.3–1.77)	0.8 (0.4–2.3)	0.7 (0.3–1.9)	0.040	0.018
nGH	0.6 (0.3–2.4)	0.3 (0.12–0.65)	0.3 (0.1–0.8)	0.3 (0.1–0.7)	0.335	0.140
IGF-1 (ng/mL)	314 (198–551)	242 (164–394)	278 (184–416)	248 (174–399)	0.201	0.138
ULNR-IGF-1 (%)	128 (79.6–210)	100 (66.3–155)	114 (82.4–175)	104 (74.4–161)	0.317	0.217
IGFBP3 (mg/L)	5.1 ± 1.4	4.9 ± 1.3	5.5 ± 1.7	5.1 ± 1.4	0.074	0.970

Values are expressed as mean ± SD (range)

DM diabetes mellitus, bGH baseline GH, nGH nadir GH, ULNR upper limit of the normal age- and sex-matched range

<sup>a</sup> Additive (or co-dominant) model: C/C × A/C × A/A

<sup>b</sup> Dominant model: C/C × (A/C + A/A)

<sup>c</sup> Serum GH data are log10 transformed

The relationship between GH and IGF1 levels appear to be linear in patients with active disease [1, 2], but discrepant results may be observed in many acromegalic patients either at diagnosis either after treatment [3–8]. Bianchi et al. [22] described that treated acromegalic patients carrying the GHR-d3 allele were likely to have post treatment GH values lower than those fl/fl homozygous but with an increased rate of GH and IGF-I levels discordancy (high IGF-I levels and GH ≤2 ng/ml) after SA [22]. On the other hand, Kamenicky et al. [24] observed that the GHR-exon 3 genotype did not affect the positive relationship between GH and IGF-I levels. In our cohort of acromegalic patients, the discrepancy between GH and IGF-1 levels at diagnosis, after surgery and after medical treatment did not correlate to GHR-exon3 genotypes.

There are also contrasting findings about GHR-d3 polymorphism influence on the outcome of acromegaly treatment. Mercado et al. [17] observed that subjects carrying GHR-fl allele had greater probability of achieving IGF-I normalization after either surgical or medical therapy. In other two studies, GHR-d3 allele was associated to lower pegvisomant dose of and fewer months of treatment to normalize IGF-I [23, 26]. In contrast, another study

reported that GHR-d3 was not a predictor of both dose and efficacy of mono- and combined pegvisomant therapies in 111 patients with acromegaly [27]. In our cohort, the post-treatment GH levels were not significantly lower in patients carrying the GHR-d3 despite similar IGF-1 levels. Also, no influence of GHR-exon 3 genotypes on treatment outcome was found in our study. The mean number of treatments to which patients underwent was similar among the GHR-d3 genotypes. This is in contrast with previous studies that observed a higher number of treatments in GHR-d3 compared to GHR-fl genotype [17, 25].

In addition, it has been hypothesized that patients who carried GHR-d3 would be more susceptible to long-term complications of acromegaly. Mercado et al. [17] demonstrated an increased prevalence of type 2 DM in active acromegaly patients with the d3 genotype [17]. Wassenaar et al. [28] observed that the GHR-d3 was associated with a higher prevalence of colonic polyps and dolichocolon and osteoarthritis and Turgut et al. [29] observed that systolic blood pressure was significantly increased in homozygote GHR-d3 genotype group compared to d3/fl subjects. However, our results did not find any consistent results on percentages of patients with comorbidities among GHR-exon 3 genotypes.

**Table 5** Summary of studies on the impact of GHR-exon 3 polymorphism in acromegalic patients

References	Patients		Effect of GHR genotype on:	GHR genotype %			Significant influence of GHR-d3 versus GHR-fl genotypes
	No.	conditions		fl/fl	fl/d3	d3/d3	
<i>At diagnosis</i>							
Schmid et al. [16]	44	Untreated	Relationship between GH and IGF1	50	41	9	GHR-d3 had lower GH levels than those GHR-fl, but comparable IGF-I levels
Kamenicky et al. [24]	105	Untreated	Relationship between GH and IGF1	51	30	19	GHR-d3 was significantly younger than fl/fl. GHR-d3 was associated with a more morbid acromegalic clinical and biochemical picture. GHR-d3 genotype did not affect the positive relationship between GH and IGF-I levels
Turgut et al. [29]	35	Untreated	Clinical features and comorbidities	54	31	14	GHR-d3 showed significantly increased in systolic blood pressure, BMI, fasting insulin and HOMA-IR
<i>At diagnosis/after treatment</i>							
Mercado et al. [17]	148	Surg, RT, DA, AS	Clinical and biochemical features, comorbidities and treatment outcome	45	32	22	GHR-d3 may be associated with slightly higher prevalence of diabetes mellitus After treatment, GHR-d3 had lower chance of achieving IGF-I normalization with SA and an increased rate of GH and IGF-I discrepancy GHR-d3 genotypes required higher number of treatments
Bianchi et al. [22]	84	Surgery, RT,	Relationship between GH and IGF1	52	30	18	GHR-d3 allele were likely to have post treatment GH values lower than those fl/fl homozygous but with an increased rate of GH and IGF-I levels discordant (high IGF-I levels and GH $\leq 2$ ng/ml) after SA
Bianchi et al. [23]	19	Surgery, AS, PEG	Efficacy of PEG	53	31	16	GHR-d3 showed better response PEG treatment, lower PEG doses and fewer months to normalize IGF-I
Wassenaar et al. [28]	86	Biochemical remission (after surg, RT or on AS)	Clinical features and comorbidities	59	34	7	GHR-d3 was associated with an increased prevalence of osteoarthritis, dolichocolon, and adenomatous colonic polyps
Bernabeu et al. [26]	44	Surgery, RT, AS, PEG	Efficacy of PEG	41	57	2	GHR-d3 showed better response PEG treatment, lower PEG doses and fewer months to normalize IGF-I
Montefusco et al. [25]	76	Surg, RT, DA, AS, PEG	Clinical (comorbidities)	55	35	9	GHR-d3 had lower BMI. GHR-d3 genotypes required higher number of treatments
Filopanti et al. [27]	127	Sur, RT, AS, PEG	Efficacy of PEG	68	41	18	GHR-d3 did not showed better response PEG treatment
Present study	186	Surg, RT, DA, AS,	Clinical and biochemical features, comorbidities and treatment outcome	45	44	22	GHR-d3 did not influence clinical and laboratorial features at diagnosis and after treatment

*Sur* surgery, *RT* radiotherapy, *SA* somatostatin analogs, *DA* dopamine agonists, *PEG* pegvisomant

IGFBP3 displays growth inhibitory/proapoptotic action and counteracts the IGF-1 tumor-promoting effects by down regulating its bioavailability [21]. To our knowledge, only one study has evaluated the role of IGFBP-3 polymorphisms in acromegaly to date. Akin et al. [30] examined 34 patients with acromegaly and 37 healthy subjects and compared -202 A/C IGFBP3 genotypes with serum levels of glucose, insulin, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, growth hormone (GH),

Insulin-like growth factor I (IGF-I) and IGFBP-3 [30]. They observed that the frequency of -202 A/C IGFBP3 genotypes was significantly different between control and patients but with no difference in the clinical presentation of acromegalic patients [30]. In our evaluation, no association between -202 A/C IGFBP3 and clinical/hormonal characteristics and response to therapy was found, with the exception of GH levels after treatment that were significantly lower in patients carrying A allele.

In conclusion, our results did not find any consistent evidence for the influence of GHR-exon 3 or -202 A/C IGFBP3 polymorphisms in the variability on phenotypic expression of GH hypersecretion in terms of clinical features, co-morbidities and response to treatment and its peripheral biological effects in patients with acromegaly.

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**Conflict of interest** The authors declare no conflict of interest.

## References

- Dobrashian RD, O'Halloran DJ, Hunt A, Beardwell CG, Shalet SM (1993) Relationships between insulin-like growth factor-1 levels and growth hormone concentrations during diurnal profiles and following oral glucose in acromegaly. *Clin Endocrinol (Oxf)* 38:589–593
- Parkinson C, Ryder WD, Trainer PJ, Group SAS (2001) The relationship between serum GH and serum IGF-I in acromegaly is gender-specific. *J Clin Endocrinol Metab* 86:5240–5244
- Dimaraki EV, Jaffe CA, DeMott-Friberg R, Chandler WF, Barkan AL (2002) Acromegaly with apparently normal GH secretion: implications for diagnosis and follow-up. *J Clin Endocrinol Metab* 87:3537–3542
- Freda PU, Nuruzzaman AT, Reyes CM, Sundeen RE, Post KD (2004) Significance of “abnormal” nadir growth hormone levels after oral glucose in postoperative patients with acromegaly in remission with normal insulin-like growth factor-I levels. *J Clin Endocrinol Metab* 89:495–500
- Mercado M, Espinosa de los Monteros AL, Sosa E et al (2004) Clinical–biochemical correlations in acromegaly at diagnosis and the real prevalence of biochemically discordant disease. *Horm Res* 62:293–299
- Alexopoulou O, Bex M, Abs R, T'Sjoen G, Velkeniers B, Maiter D (2008) Divergence between growth hormone and insulin-like growth factor-I concentrations in the follow-up of acromegaly. *J Clin Endocrinol Metab* 93:1324–1330
- Machado EO, Taboada GF, Neto LV et al (2008) Prevalence of discordant GH and IGF-I levels in acromegalics at diagnosis, after surgical treatment and during treatment with octreotide LAR. *Growth Horm IGF Res* 18:389–393
- Ho PJ, Jaffe CA, Friberg RD, Chandler WF, Barkan AL (1994) Persistence of rapid growth hormone (GH) pulsatility after successful removal of GH-producing pituitary tumors. *J Clin Endocrinol Metab* 78:1403–1410
- Walenkamp MJ, Wit JM (2006) Genetic disorders in the growth hormone—insulin-like growth factor-I axis. *Horm Res* 66:221–230
- Pantel J, Machinis K, Sobrier ML, Duquesnoy P, Goossens M, Amselem S (2000) Species-specific alternative splice mimicry at the growth hormone receptor locus revealed by the lineage of retroelements during primate evolution. *J Biol Chem* 275:18664–18669
- Buzi F, Mella P, Pilotta A, Prandi E, Lanfranchi F, Carapella T (2007) Growth hormone receptor polymorphisms. *Endocr Dev* 11:28–35
- Dos Santos C, Essieux L, Teinturier C, Tauber M, Goffin V, Bougnères P (2004) A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. *Nat Genet* 36:720–724
- Sobrier ML, Duquesnoy P, Duriez B, Amselem S, Goossens M (1993) Expression and binding properties of two isoforms of the human growth hormone receptor. *FEBS Lett* 319:16–20
- Urbanek M, Russell JE, Cooke NE, Liebhaber SA (1993) Functional characterization of the alternatively spliced, placental human growth hormone receptor. *J Biol Chem* 268:19025–19032
- Bass SH, Mulkerrin MG, Wells JA (1991) A systematic mutational analysis of hormone-binding determinants in the human growth hormone receptor. *Proc Natl Acad Sci USA* 88:4498–4502
- Schmid C, Kraysenbuehl PA, Bernays RL, Zwimpfer C, Maly FE, Wiesli P (2007) Growth hormone (GH) receptor isoform in acromegaly: lower concentrations of GH but not insulin-like growth factor-1 in patients with a genomic deletion of exon 3 in the GH receptor gene. *Clin Chem* 53:1484–1488
- Mercado M, González B, Sandoval C et al (2008) Clinical and biochemical impact of the d3 growth hormone receptor genotype in acromegaly. *J Clin Endocrinol Metab* 93:3411–3415
- Costalonga EF, Antonini SR, Guerra-Junior G, Mendonca BB, Arnhold IJ, Jorge AA (2009) The -202 A allele of insulin-like growth factor binding protein-3 (IGFBP3) promoter polymorphism is associated with higher IGFBP-3 serum levels and better growth response to growth hormone treatment in patients with severe growth hormone deficiency. *J Clin Endocrinol Metab* 94:588–595
- van der Kaay DC, Hendriks AE, Ester WA et al (2009) Genetic and epigenetic variability in the gene for IGFBP-3 (IGFBP3): correlation with serum IGFBP-3 levels and growth in short children born small for gestational age. *Growth Horm IGF Res* 19:198–205
- Jernström H, Deal C, Wilkin F et al (2001) Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in healthy premenopausal women. *Cancer Epidemiol Biomarkers Prev* 10:377–384
- Deal C, Ma J, Wilkin F et al (2001) Novel promoter polymorphism in insulin-like growth factor-binding protein-3: correlation with serum levels and interaction with known regulators. *J Clin Endocrinol Metab* 86:1274–1280
- Bianchi A, Giustina A, Cimino V et al (2009) Influence of growth hormone receptor d3 and full-length isoforms on biochemical treatment outcomes in acromegaly. *J Clin Endocrinol Metab* 94:2015–2022
- Bianchi A, Mazziotti G, Tilaro L et al (2009) Growth hormone receptor polymorphism and the effects of pegvisomant in acromegaly. *Pituitary* 12:196–199
- Kamenicky P, Dos Santos C, Espinosa C et al (2009) D3 GH receptor polymorphism is not associated with IGF1 levels in untreated acromegaly. *Eur J Endocrinol* 161:231–235
- Montefusco L, Filopanti M, Ronchi CL et al (2010) d3-Growth hormone receptor polymorphism in acromegaly: effects on metabolic phenotype. *Clin Endocrinol (Oxf)* 72:661–667
- Bernabeu I, Alvarez-Escolá C, Quinteiro C et al (2010) The exon 3-deleted growth hormone receptor is associated with better response to pegvisomant therapy in acromegaly. *J Clin Endocrinol Metab* 95:222–229
- Filopanti M, Olgiati L, Mantovani G et al (2012) Growth hormone receptor variants and response to pegvisomant in monotherapy or in combination with somatostatin analogs in acromegalic patients: a multicenter study. *J Clin Endocrinol Metab* 97:E165–E172
- Wassenaar MJ, Biermasz NR, Pereira AM et al (2009) The exon-3 deleted growth hormone receptor polymorphism predisposes to long-term complications of acromegaly. *J Clin Endocrinol Metab* 94:4671–4678

29. Turgut S, Akin F, Ayada C, Topsakal S, Yerlikaya E, Turgut G (2012) The growth hormone receptor polymorphism in patients with acromegaly: relationship to BMI and glucose metabolism. *Pituitary* 15:374–379
30. Akin F, Turgut S, Cirak B, Kursunluoglu R (2010) IGF(CA)19 and IGFBP-3-202A/C gene polymorphism in patients with acromegaly. *Growth Horm IGF Res* 20:399–403