# Influence of Growth Hormone Receptor d3 and Full-Length Isoforms on Biochemical Treatment Outcomes in Acromegaly

Antonio Bianchi, Andrea Giustina, Vincenzo Cimino, Roberto Pola, Flavia Angelini, Alfredo Pontecorvi, and Laura De Marinis

Division of Endocrinology (A.B., V.C., A.P., L.D.M.) and Internal Medicine (R.P., F.A.), Catholic University School of Medicine, 00168 Rome, Italy; and Department of Medical and Surgical Sciences (A.G.), University of Brescia, 25121 Brescia, Italy



**Context:** In acromegaly, a discrepancy between what are defined as "normal" levels of GH and IGF-I for every given patient is observed in up to 35% of subjects at diagnosis and during the follow-up.

**Objective:** The aim of the study was to evaluate the impact of GH receptor (GHR) polymorphism on the biochemical assessment of the treatment of acromegaly and on prevalence of discordant levels of GH and IGF-I.

**Setting:** The study was performed in an institutional referral center at a tertiary care hospital.

**Design, Patients, and Methods:** We studied prospectively and retrospectively 84 consecutive acromegalic patients with active disease after neurosurgery and treated them with somatostatin analogs. The GHR genotype (flfl, fld3, or d3d3) was determined from peripheral blood.

**Results:** Lack of exon 3 of GH receptor (d3-GHR) was found in 40 of 84 patients (47.6%). After neurosurgery, 67 subjects (79.8%) of the study population, concordant active acromegalic patients, had high IGF-I and mean GH levels above 2 ng/ml, whereas the remaining 17 patients (20.2%, discordant active acromegalic patients) showed discordance between these two parameters (high IGF-I and GH levels $\leq$ 2 ng/ml). Overall, 70.6% of discordant patients were carriers of the d3-GHR. After somatostatin analogs, discordant active acromegalic patients increased to 30.9%, 69.2% of whom were carriers of the d3-GHR. Logistic regression analysis demonstrated that d3-GHR carriers maintained the significant correlation with discordant GH and IGF-I values either after neurosurgery or after somatostatin analog treatment, independently of the effects of age, sex, duration of acromegaly, serum GH, and IGF- I values either at diagnosis of acromegaly or after neurosurgery.

**Conclusion:** The GHR polymorphism seems to have a relevant impact on the posttreatment biochemical assessment of acromegaly. Moreover, the d3-GHR isoform could be an independent predictor of GH and IGF-I discrepancy during the follow-up in acromegaly. **(*J Clin Endocrinol Metab* 94: 2015–2022, 2009)**

The GH receptor (GHR) is a single peptide chain with extracellular hormone-binding, transmembrane, and cytoplasmic domains (1). The GHR is expressed in many tissues and mediates the effects of GH on body growth, intermediary metabolism, and cell differentiation (2, 3). The GHR gene consists of nine exons encoding the receptor (4) and several untranslated exons (4, 5). Two mRNA transcripts, differing in the coding part, have been identified for the human GHR, one containing (GHR3+) and one lacking (GHR3−) exon 3 (6, 7).

Approximately half of the individuals in most populations tested were shown to be carriers of the d3-GHR. Initial studies comparing d3-GHR and full-length (fl)-GHR indicated no differences in GH binding, but subsequent analysis using reporter constructs to assess transcriptional activity showed that deletion

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2008-1337 Received June 23, 2008. Accepted March 19, 2009.
First Published Online March 31, 2009

Abbreviations: CI, Confidence interval; fl, full length; GHR, GH receptor; NS, neurosurgery; SDS, SD score; SSA, somatostatin analog.

**TABLE 1.** Clinical and anthropometric characteristics of the entire study population and according to GHR haplotypes

| | | | d3-carriers | | |
|---|---|---|---|---|---|
| | **Total patients** | **flfl** | **fld3** | **d3d3** | **(fld3 + d3d3)** |
| Patients (n) | 84 | 44 | 25 | 15 | 40 |
| Age (yr) | 48.5 (24–86) | 44.5 (24–86) | 50 (29–77) | 47 (29–66) | 49.5 (29–77) |
| BMI (kg/m$^2$) | 25.7 (18.82–41.03) | 26.3 (19.53–41.03) | 25.4 (18.8–37.66) | 23.9 (20.9–32.03) | 24.9 (18.82–37.76) |
| Males (n) | 34 | 17 | 8 | 9 | 17 |
| Females (n) | 50 | 27 | 17 | 6 | 23 |
| Duration of disease (yr) | 10 (4–30) | 10 (4–30) | 10 (4–30) | 10 (6–15) | 10 (4–30) |
| Macroadenomas (n) | 67 | 35 | 20 | 13 | 33 |
| Microadenomas (n) | 17 | 9 | 5 | 2 | 7 |

The results are shown as median (range). BMI, Body mass index; flfl, patients with the flfl haplotype, homozygous for the full length GHR isoform; d3-carriers, patients homo- or heterozygous for the exon 3 lacking isoforms; fld3, patients with the fld3 haplotype, heterozygous for the exon 3 lacking isoform; d3d3, patients with the d3d3 haplotype, homozygous for the exon 3 lacking isoform.

of exon 3 affected the functional properties of the GHR (8). Moreover, young GH-deficient carriers of an allele encoding the d3-GHR were more responsive (in terms of growth) to GH administration than homozygous fl-GHR (8). This finding was confirmed in children with Turner syndrome, those with short-stature small-for-gestational-age, and those with severe GHD (9, 10), but not in children with short-stature non-GH-deficient small-for-gestational-age and those with short stature appropriate for gestational age or isolated GH deficiency (11–13). In healthy individuals, differences in GH sensitivity may be compensated for by adjusted GH secretion.

Recently, 50% of patients with acromegaly were shown to have at least one d3 allele and lower GH levels for any given serum IGF-I concentration compared with fl-GHR homozygous carriers (14). Moreover, patients with acromegaly carrying the d3 isoform of the GHR were found to be at risk of more severe complications as well as more difficult biochemical control (15). Acromegaly is a potentially very severe disease because chronic GH hypersecretion may cause cardiometabolic complications finally leading to decreased life expectancy. With availability of modern biochemical techniques as well as effective therapeutic tools, progression of acromegaly can be blocked in its early stages (16). Because morbidity and mortality in acromegaly normalize with tight biochemical control, a rigorous biochemical assessment of acromegaly after any treatments is mandatory (17, 18).

Although a combined evaluation of IGF-I and GH response to the oral glucose tolerance test is currently thought to provide the most reliable assessment of biochemical status in acromegaly, several pitfalls in the interpretation of either test have been reported (19–21) and discrepant results may be observed (22–24). In a few cases, abnormal pulsatile GH secretion, which suggests disordered GH neuroregulation, can persist after surgery despite normal IGF-I levels (25); the opposite pattern of divergent GH and IGF-I results has been more frequently reported, with apparently "normal" (<2.5 ng/ml) mean GH levels from day curves or frequent 24-h sampling and elevated IGF-I levels being reported in up to 35% of patients with acromegaly (26–29). Reasons for these discrepancies are still unclear and under current active investigations (27).

We evaluated the impact of GHR polymorphism on the biochemical assessment of acromegaly treatment and prevalence of discordant GH and IGF-I levels at diagnosis and during follow-up.

## Subjects and Methods

### Subjects

We studied a cohort of 84 consecutive acromegalic patients with elevated IGF-I levels (*i.e.* with active disease) after neurosurgery. The median age of the patients was 48.5 yr (range, 24–86). Acromegaly was previously diagnosed based on medical history, clinical examination, and failure of suppression of serum GH concentrations below 1 ng/ml after a 75-g oral glucose load and fasting plasma IGF-I concentrations above the normal ranges for sex and age. The median duration of disease before surgery, estimated on the basis of clinical history, *i.e.* when the patient recalled appearance of signs and symptoms of the disease, was 10 yr (range, 4–30). Three months after neurosurgery, serum IGF-I and GH day curve (0, 30, 60, 90, and 120 min) were evaluated. GH levels were defined as normal when the average of the GH day curve was no greater than 2 ng/ml. All the patients, independently of either GH levels or evidence at magnetic resonance imaging of persistent pituitary mass, were treated with somatostatin analogs (SSAs). Biochemical reevaluation with GH day curve and IGF-I levels was performed in all the patients 6 months after beginning medical therapy. Anthropometric and clinical characteristics of the patients are reported in Table 1. At study entry and during the follow-up, all acromegalic patients were classified according to disease activity and concordant or discordant GH and IGF-I serum levels as: 1) patients with active acromegaly, high IGF-I levels for age and sex, and GH serum levels above 2 ng/ml (concordant active acromegalic patients); 2) patients with active acromegaly, high IGF-I levels for age and sex, and GH serum levels no greater than 2 ng/ml (discordant active acromegalic patients); 3) patients with controlled acromegaly, normalized IGF-I levels for age and sex, and GH serum levels above 2 ng/ml (discordant controlled acromegalic patients); and 4) patients with controlled acromegaly, normalized IGF-I levels for age and sex, and GH serum levels no greater than 2 ng/ml (concordant controlled acromegalic patients). Baseline and postneurosurgery and postmedical treatment parameters are reported in Table 2.

The patients gave informed consent to the study that was approved by the local Ethical Committee.

### Biochemical measurements

Blood samples were collected after an overnight fasting. Serum was promptly separated and stored at −20 C until assay. GH and IGF-I were measured by Immulite 2000 (Diagnostic Products Corp., Los Angeles, CA). The interassay coefficients of variation of GH and IGF-I assays ranged from 5.5 to 6.2% and from 6.4 to 11.5%, respectively. Sensitivity limits of the assays were 0.01 and 0.2 ng/ml, respectively. The concentration of IGF-I was also expressed as SD score (SDS), using the corresponding reference values, expressed in relation to normal age-adjusted adult values (normal values range from −2 to + 2 SDS) calculated as follows: SDS = (in-value − mean of normal age-adjusted values)/(SD of mean of normal age-adjusted values) (30).

**TABLE 2.** Biochemical findings in the entire study population and according to GHR haplotypes at diagnosis, after neurosurgery, and after SSA treatment

| | | | d3-carriers | | |
|---|---|---|---|---|---|
| | Total patients | flfl | fld3 | d3d3 | fld3 + d3d3 |
| Patients (n) | 84 | 44 | 25 | 15 | 40 |
| GH at diagnosis (ng/ml) | 19 (1.8–89) | 23 (1.8–78) | 17 (4–89) | 17.4 (6.6–76) | 17.2 (4–89) |
| GH after NS (ng/ml) | 2.96 (0.7–64)[a] | 3.9 (1.1–64)[a] | 2.5 (0.72–16.2) | 2.9 (1–14.4) | 2.8 (0.72–16.2)[a,c] |
| GH after SSAs (ng/ml) | 0.8 (0.03–25)[a,b] | 0.95 (0.03–25)[a,b] | 0.5 (0.1–11.8) | 0.65 (0.06–2) | 0.6 (0.06–11.8)[a,b] |
| IGF-I at diagnosis (ng/ml) | 759 (345–1561) | 743.5 (390–1561) | 761 (378–1377) | 664 (345–1179) | 759 (345–1377) |
| SDS at diagnosis | 10.8 (3.9–34.4) | 9.2 (4.1–34.4) | 12.53 (4.2–26.8) | 11.5 (3.9–17.9) | 11.6 (3.9–26.8) |
| IGF-I after NS (ng/ml) | 469.5 (276–1480)[a] | 474.5 (276–1480)[a] | 396 (259–872) | 477 (322–864) | 460 (315–872)[a] |
| SDS after NS | 5.6 (1.9–32.4) | 5.7 (2.5–32.4) | 4.9 (1.9–16) | 6.1 (2.9–11.5) | 5.1 (1.9–16) |
| IGF-I after SSAs (ng/ml) | 237.5 (90–1058)[a,b] | 232.5 (90–1058)[a,b] | 256 (127–772) | 250 (119–559) | 253 (119–772)[a,b] |
| SDS after SSAs | 1.5 (−1.0–18.8) | 1.4 (−1.0–18.8) | 2.2 (−0.6–11.5) | 1.6 (0.1–6.3) | 1.76 (−0.6–2.5) |

The results are shown as median (range). Conversion factors to SI units: GH, × 2.225 = mU/liter; IGF-I, × 0.131 = nmol/liter. GH at diagnosis represents GH nadir after oral glucose tolerance test; GH after NS and after SSAs represent mean value of GH day curve. IGF-I are also expressed as SDS. NS, Neurosurgery.

[a] $P < 0.05$ *vs.* at diagnosis.

[b] $P < 0.05$ *vs.* after NS.

[c] $P < 0.05$ *vs.* flfl.

### Genetic testing

DNA was extracted from 100/200 $\mu$g/liter peripheral bloom by Kit Illustra blood Genomic Prep Mini Spin (GE Healthcare, Little Chalfont, United Kingdom) (for the rapid and reproducible isolation of high-quality genomic DNA from whole blood) for the detection of the GHR gene polymorphism.

Polymorphisms were studied through PCR by Eppendorf Master Mix (2.5×) (Eppendorf, Hamburg, Germany). [The 2.5× Eppendorf Master Mix contains Taq DNA polymerase (0.0625 U/$\mu$l), 125 mM KCl, 75 mM Tris-HCl (pH 8.3), 3.75 mM $MgCl_2$, 0.25% Nonidet-P40, 500 $\mu$M of each dNTP, and stabilizers. This corresponds to final concentrations in the PCR of 1.25 U Taq DNA polymerase, 50 mM KCl, 30 mM Tris-HCl, 1.5 mM $Mg^{2+}$, and 200 $\mu$M of each dNTP]. Amplification was performed using primers, as described previously (31); 5′ TGTGCTGGTCTGTTGGTCTG 3′, 5′ AGTCGGTTCCTGGGACGAGA 3′, and 5′ CCTGGATTAA-CACTTTGCAGACTC 3′ were treated as follows: one cycle at 94 C for 5 min (denaturing), 35 cycles at 94 C for 30 sec (denaturing) and 60 C for 30 sec (annealing), 72 C for 60 sec (extension), and one cycle at 72 C for 7 min (last extension). The amplification products were separated by electrophoresis on 2% agarose gel and visualized with the use of ethidium bromide. The full-length allele (GHRfl) is represented by a 935-bp fragment and the exon 3-deleted allele (GHRd3) by a 532-bp fragment (Fig. 1).

### Statistical analysis

Data are presented as median and range, unless otherwise stated. Unpaired data were compared using Mann-Whitney's test, whereas Friedman test was used to compare paired data. Pearson's coefficient was calculated to assess the correlation between GH and IGF-I in patients with different GHR haplotypes (*i.e.* flfl *vs.* d3 carriers). Multivariate logistic regression analysis was performed to assess the correlation between GHR haplotype and discordant response of GH and IGF-I values after neurosurgery and SSAs, independently of the effects of age, sex, duration of acromegaly, baseline serum GH and IGF-I. In the analysis of correlation between GHR haplotype and post-SSAs discordance, GH and IGF-I values after neurosurgery were also considered as covariates. Statistical significance was assumed when $P$ was less than 0.05. Based on previous data in the literature (14, 15) and on the similar pattern of response observed by us, statistical calculations were performed comparing flfl patients *vs.* d3 carriers taken as a whole. However, specific data concerning fld3 and d3d3 patients have been reported in Tables 1–3 and Fig. 2.

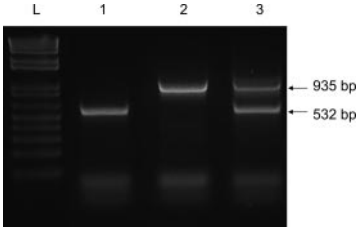


**FIG. 1.** Agarose gel electrophoresis of PCR products showing the size marker (L), d3-GHR (lane 1), fl-GHR (lane 2), and fld3-GHR (lane 3).

## Results

### Clinical characteristics

Study patients included both females and males with elevated postsurgical IGF-I levels. Median age at diagnosis of acromegaly was 48.5 yr (range, 24–86), and body mass index was 25.67 kg/m$^2$ (range, 18.82–41.03). Acromegalic symptoms had been present for 10 yr (range, 4–30). Before neurosurgery, magnetic resonance imaging revealed macroadenomas (>10 mm in diameter) in 67 patients (79.7%) and microadenomas in 17 (20.3%).

### Genomic analysis

We found 44 (52.4%) patients to be homozygous for fl-GHR and 40 (47.6%) patients to be carriers of at least one allele of the d3-GHR isoform (15 homozygous for the d3 isoform). Patients were assigned to three groups: carriers homozygous for the fl-GHR (flfl-GHR group), heterozygous carriers of the d3-GHR (fld3 group), and homozygous carriers of the d3-GHR (d3d3-GHR group). Demographic and clinical characteristics at diagnosis did not differ significantly in the three groups (Table 1).

### Hormonal data

#### *Baseline*

At the time of diagnosis of acromegaly, no significant differences in serum GH ($P = 0.5$) and IGF-I ($P = 0.6$) concentrations were observed between the flfl-GHR group and patients harboring d3 isoform (Table 2).

**TABLE 3.** Outcome of acromegalic patients and prevalence of discordance between GH and IGF-I, according to GHR haplotypes

| | Total patients | flfl | d3-carriers | | |
|---|---|---|---|---|---|
| | | | fld3 | d3d3 | fld3+d3d3 |
| No. of patients | 84 | 44 | 25 | 15 | 40 |
| After neurosurgery | | | | | |
| Concordant active acromegalic patients | 67 (79.8) | 39 (88.6) | 17 (68) | 11 (73.3) | 28 (70) |
| Discordant active acromegalic patients | 17 (20.2) | 5 (11.4) | 8 (32) | 4 (26.7) | 12 (30)[a] |
| Discordant controlled acromegalic patients | | | | | |
| After SSAs | | | | | |
| Concordant controlled acromegalic patients | 45 (53.6) | 24 (54.5) | 12 (48) | 9 (60) | 21 (52.5) |
| Concordant active acromegalic patients | 11 (13.1) | 10 (22.8) | 1 (4) | | 1 (2.5) |
| Discordant active acromegalic patients | 26 (30.9) | 8 (18.2) | 12 (48) | 6 (40) | 18 (45)[a] |
| Discordant controlled acromegalic patients | 2 (2.4) | 2 (4.5) | | | |

Data represent number or number (percent).

[a] $P < 0.05$ *vs.* flfl.

### *After surgery*

After surgery, GH and IGF-I levels were decreased in all patients regardless of the haplotype (Fig. 2A). However, GH levels were significantly ($P = 0.04$) lower in the patients who were carriers of d3-GHR isoform as compared with the homozygous fl-GHR group, without any significant ($P = 0.22$) differences in serum IGF-I SDS values (Table 2).

After neurosurgery, 67 (79.8%) patients showed concordant serum GH and IGF-I values, whereas discordant values (*i.e.* "safe" GH values and high IGF-I SDS values in all discordant cases) were observed in 17 patients (20.2%), the prevalence being significantly higher in the carriers of d3-GHR as compared with flfl haplotype (30 *vs.* 11.4%; $\chi^2$, 4.5; $P = 0.03$). Overall, 12 of 17 (70.6%) discordant patients and 28 of 67 (41.8%) concordant patients were carriers of the d3-GHR (Table 3).

### *After medical treatment*

After SSA treatment, IGF-I levels were decreased in all patients ($P < 0.001$ *vs.* after neurosurgery values) (Fig. 2B), also independently of GHR haplotype groups, and similar IGF-I levels were found in the two groups ($P = 0.8$). A trend toward lower GH levels ($P = 0.2$) was again observed in the d3-GHR carriers compared with the fl-GHR group (Fig. 2B and Table 2).

Fifty-six patients had concordant serum GH and IGF-I values, whereas discordant values were observed in 28 patients (Table 3). Twenty-six (30.9%) patients had "safe" GH and high IGF-I SDS values, whereas in two (2.4%) discordant patients, serum GH values were high with normal serum IGF-I SDS. The prevalence of discordant safe GH and high IGF-I values was significantly higher in the carriers of d3 haplotype as compared with flfl haplotype (45 *vs.* 18.2%; $\chi 2, 6.4$; $P = 0.01$). Overall, 18 of 26 (69.2%) discordant patients and 22 of 56 (39.3%) concordant patients were carriers of d3-haplotype (Table 3). Of the 17 patients with discordant GH and IGF-I levels after neurosurgery, five became concordant, whereas 12 maintained discordant values after SSA treatment. Moreover, 14 patients with concordant GH and IGF-I values after neurosurgery became discordant after SSAs. The GHR haplotype was not significantly correlated with the change in GH-IGF-I concordance after SSAs treatment (data not shown).

### Correlations

The relationship between serum GH concentrations and IGF-I SDS concentrations in the three groups at baseline, after surgery, and after medical treatment is shown in Fig. 3. Similar correlations were found with presurgical data in the two groups. After surgery and medical treatment, the curves were shifted to the left in the d3 carriers with a significant change in the slope with respect to the fl-GHR group. In fact, at any given serum IGF-I concentration, serum GH concentrations were lower in carriers of the d3-GHR compared with patients carrying only the fl-GHR isoform. In addition, for a given GH concentration, carriers of the d3-GHR had higher IGF-I concentrations compared with carriers of the fl-GHR.

### Multivariate analysis

Logistic regression analysis demonstrated that d3 haplotype maintained the significant correlation with discordant GH and IGF-I values (*i.e.* "safe" GH with and high IGF-I SDS values) either after neurosurgery [odds ratio, 4.4; 95% confidence interval (CI), 1.2–16.3; $P = 0.03$] or after SSA treatment (odds ratio, 3.7; 95% CI, 1.2–11.0; $P = 0.01$) independently of age, sex, duration of acromegaly, serum GH, and IGF- I values either at diagnosis of acromegaly or after neurosurgery (Table 4).

## Discussion

Our results show that patients with acromegaly carrying the d3-GHR isoform were likely to have posttreatment GH values lower than those carrying the homozygous fl isoform. Moreover, most of the patients showing elevated IGF-I levels in the presence of apparently normalized GH posttreatment values were carriers of at least one d3 allele. Our data suggest that a possible explanation for these findings is the higher sensitivity of the d3-GHR isoform.

Classical studies reported standardized mortality rates in patients with acromegaly to be 1.6–3.3; recent studies reported the rate to be lower, ranging from 1.3–1.8. However, when disease activity is controlled, the relative mortality risk in acromegaly is normalized. Assessment of biochemical activity of acromegaly is, however, not always unequivocal. In fact, circulating GH levels may

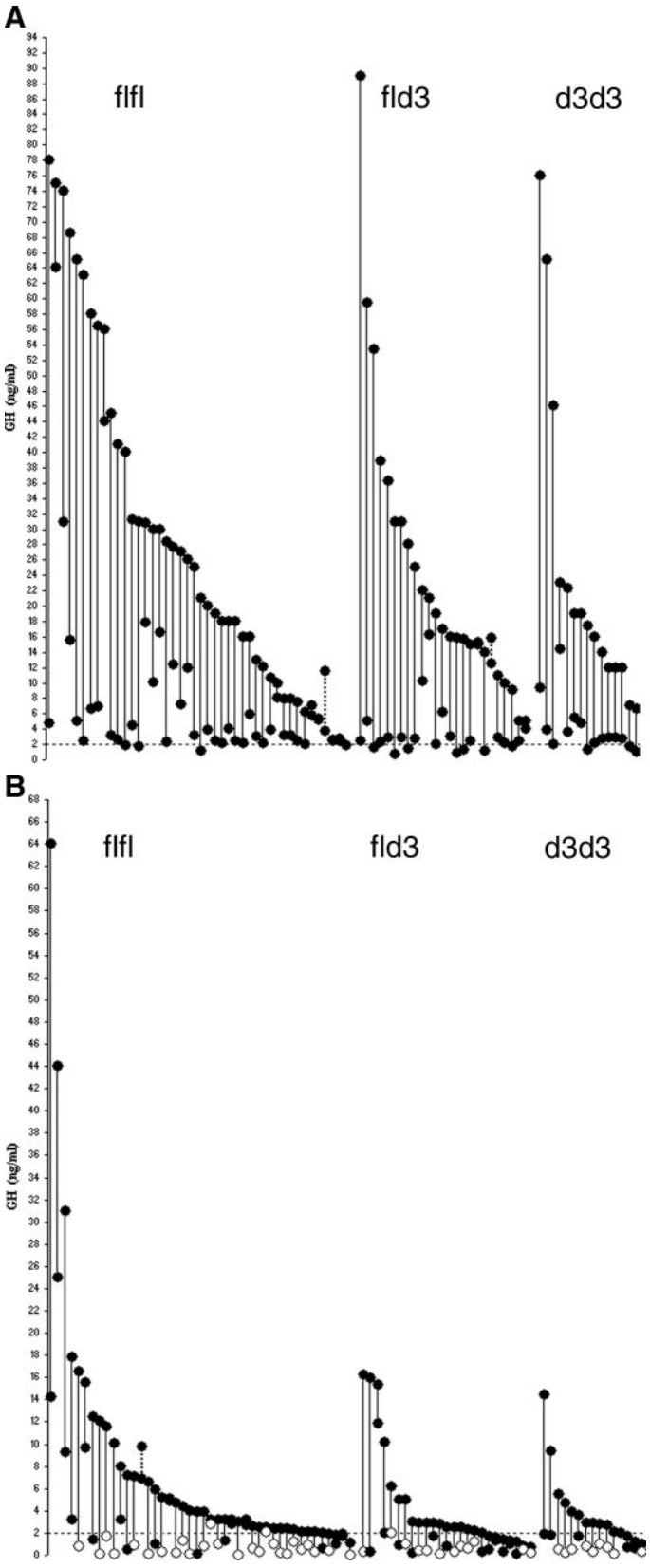


**FIG. 2.** A, Individual GH levels of 84 acromegalic patients at diagnosis and after neurosurgery according to GHR haplotypes. The *horizontal dotted line* represents the GH level of 2 ng/ml. All patients had elevated IGF-I levels for sex and age after surgery. B, Individual GH levels of 84 acromegalic patients after neurosurgery treated with SSAs, according to GHR haplotypes. The *horizontal dotted line* represents the GH level of 2 ng/ml. ●, Patients with still elevated IGF-I; ○, patients with normalized IGF-I after SSAs.

often overlap in normal subjects and patients with active and controlled acromegaly, due to the pulsatile physiological nature of GH secretion and to the short half-life of GH in plasma (32). Therefore, both dynamic testing of GH secretion (21, 33, 34) and particularly the assay of GH-dependent proteins, primarily IGF-I, has progressively integrated GH assay for biochemical assessment of acromegaly. IGF-I is a prevalently GH-regulated peptide that mediates most growth-promoting and anabolic GH actions (35–37) and is stable over time due to the nonpulsatile nature of secretion. Moreover, IGF-I levels correlate well with serum GH values in healthy and acromegalic subjects (38–46). Because the decision of administering further treatment after first line surgery is mainly based on biochemical evaluation of the disease, several consensus reports and clinical studies (21, 47, 48) consistently recommended the use of binary criteria (*i.e.* normalization of both GH and IGF-I) to assess acromegaly control, due to different physiology of GH and IGF-I, pitfalls, and variability of both GH and IGF-I assay. On the other hand, in the last few years, it has clearly emerged that in many acromegalic patients (up to 35% in some recent reports) (22–24, 49), a discrepancy may occur between what are defined as "normal" levels of GH and IGF-I.

Dos Santos *et al.* (8) first reported that a common polymorphism of the GHR gene was associated with increased responsiveness to exogenous GH. Subsequent reports confirmed these findings in children carrying the d3-GHR allele with short stature and Turner syndrome (9, 10), although other authors did not observe any differences in the response to exogenous GH based on the GHR polymorphism (11–13). Recently, it has been reported that carriers of at least one d3 allele were slightly less than 50% of both normal and acromegalic subjects (14). Moreover, lower GH values with similar IGF-I levels in d3-GHR carriers *vs.* homozygous fl-GHR were found in untreated acromegalic patients (14). Inasmuch, d3-GHR polymorphism was recently found to be associated in acromegaly with a disease clinically more severe and difficult to control (15). These findings confirmed the existence of a subpopulation of acromegalic patients with a "more sensitive" GHR.

Our working hypothesis was that in the presence of increased GH sensitivity, some patients with d3 polymorphism may present normal GH values, despite persistently elevated IGF-I levels, thereby explaining, at least in part, the commonly reported high prevalence of "so called" discrepant GH and IGF-I values. To this end, we studied a large cohort of acromegalic patients with still elevated IGF-I levels after neurosurgery, assuming that these patients still had active disease. Obviously, it cannot be excluded, even if it is unlikely, that endocrine (*i.e.* insulin resistance) or technical (accuracy of IGF-I assay) factors may have influenced patient selection. Prevalence of d3-GHR polymorphism accounted for the 45% of our entire population, similar to what was previously

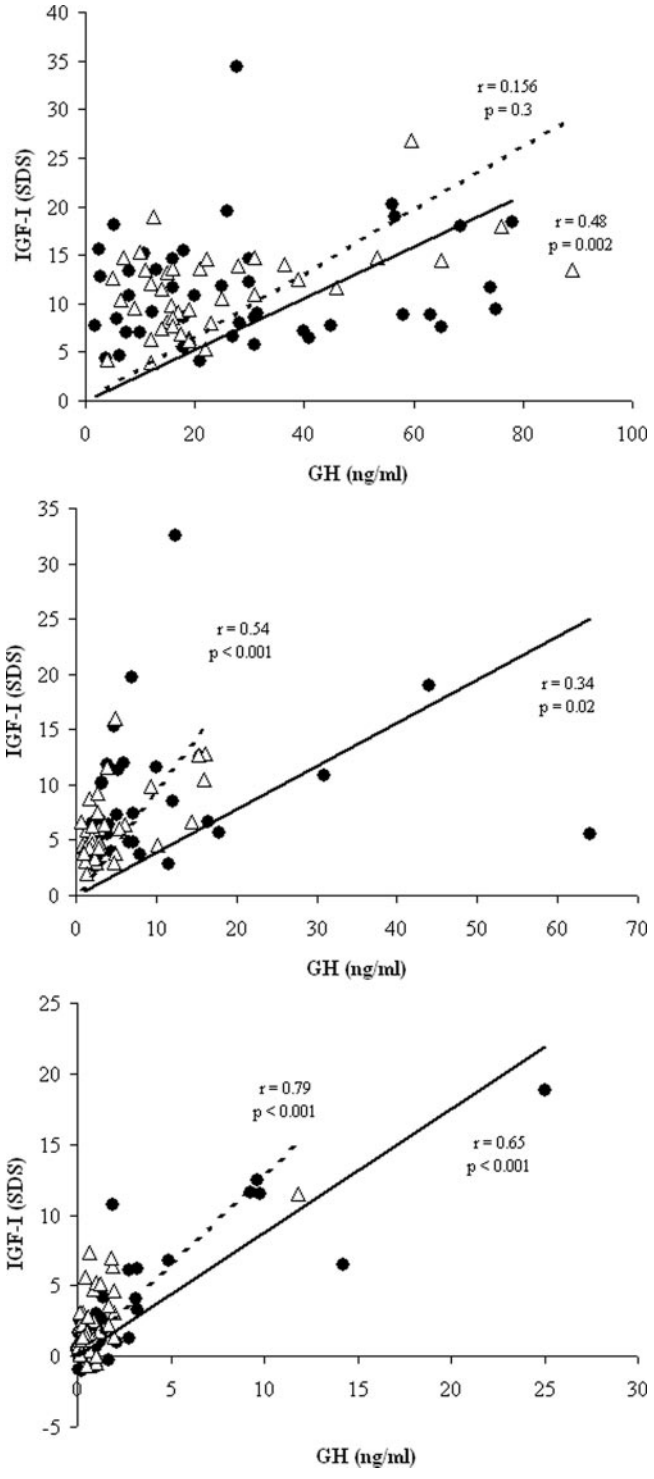


**FIG. 3.** The relationship between serum GH and IGF-I (SDS) in our patients according to GHR haplotypes at diagnosis (*upper panel*), after neurosurgery (*middle panel*), and after SSAs (*lower panel*). IGF-I levels are plotted as a function of GH levels for flfl carriers (●; *solid line*) and d3 carriers (△; *dotted line*). Regression coefficient (r) and *P* value are given in key.

reported in normal subjects as well as in smaller groups of acromegalic subjects (14, 31).

Interestingly, the presence of the d3 allele did not seem to correlate with any baseline clinical, anthropometric, and biochemical features of the subjects. This is in contrast with the only two reports published so far on GHR polymorphism in acromegaly, which suggested lower GH levels as well as more aggressive disease in d3 carriers (14, 15). This may be due in our large population to the presence of very high GH levels in some patients, which may have masked the different sensitivity of the receptor, although in our series a trend toward lower GH levels in the d3 carriers was also observed. Moreover, our study design did not enable us to confirm the finding of a more aggressive disease in d3-GHR carriers because only patients with elevated IGF-I after surgery were enrolled. However, our data demonstrated that the presence of d3 polymorphism was able to heavily influence the biochemical response in acromegaly to surgical treatment. In fact, d3 carriers did show significantly lower GH levels with respect to the subjects with the flfl-GHR, despite the presence of similar circulating IGF-I levels. This finding is in line with the hypothesis that, in the presence of GH levels closer to normal, different sensitivity of the GH receptor may be unmasked. Our data showed that, in line with previous reports, around 20% of the patients with high IGF-I levels after surgery had GH levels that according to worldwide consensus may be defined as "normal." Interestingly, 70% of these patients were carriers of the d3 allele, which suggests that even very low GH levels may retain significant biological activity in the presence of a "supersensitive" GHR. Clinically, this means that, at least in a subset of acromegalic patients, GH levels could not be a useful marker of disease activity. As a matter of fact, presurgically assessing the presence of d3 isoforms may allow an easier revaluation after surgery based only upon IGF-I assay. What are the findings that did not fit with this hypothesis? First of all, the majority of the d3-GHR patients did show concordant GH and IGF-I levels. This may mean, even among d3-GHR carriers, different sensitivity of GHR due to unknown factors, or, more likely, differences in GH sensitivity being evident

**TABLE 4.** Results of logistic regression analysis using GH-IGF-I discordance as dependent variable and d3 haplotype, age, sex, duration of disease, GH and IGF-I values at diagnosis of acromegaly as covariates

| | Odds ratio (95% CI) | *P* values |
|---|---|---|
| Discordant GH and IGF-I values after neurosurgery | | |
| d3 haplotype | 4.4 (1.2–16.3) | 0.03 |
| Age | 0.95 (0.91–1.2) | 0.10 |
| Sex | 1.8 (0.55–6.2) | 0.32 |
| Duration of acromegaly | 0.9 (0.80–1.13) | 0.48 |
| GH values at diagnosis | 0.99 (0.96–1.03) | 0.73 |
| IGF-I values at diagnosis | 0.99 (0.98–1.0) | 0.06 |
| Discordant GH and IGF-I values after SSAs | | |
| d3 haplotype | 3.7 (1.2–11.0) | 0.02 |
| Age | 1.0 (0.96–1.05) | 0.69 |
| Sex | 1.2 (0.4–3.6) | 0.67 |
| Duration of acromegaly | 0.96 (0.84–1.1) | 0.49 |
| GH values at diagnosis | 1.0 (0.98–1.03) | 0.86 |
| IGF-I values at diagnosis | 1.0 (0.98–1.0) | 0.92 |
| GH values after neurosurgery | 0.92 (0.82–1.0) | 0.21 |
| IGF-I values after neurosurgery | 1.0 (0.99–1.0) | 0.14 |

The analysis was performed at two different time-points of follow-up, *i.e.* after neurosurgery and after 6 months of SSA treatment. At this latter time-point, serum GH and IGF-I values after neurosurgery also served as covariates.

only with values very close to normal and definitely that those are the cases in which decision making is more difficult. Moreover, we found a few subjects, without the d3-GHR isoform, who showed discrepant GH and IGF-I levels; interestingly, after surgery all these patients had GH levels above 1.5 ng/ml, oppositely to d3-GHR carriers who reached GH levels of 0.5 ng/ml. This may mean that in the two subpopulations biochemical discrepancy may be related to different factors, *e.g.* variability of GH assay, as previously reported (50), in fl-GHR patients and enhanced GHR sensitivity in d3 carriers. Thus, based on our data, a cutoff of 1.5 ng/ml in the GH day curve would be consistent with discrepant values not likely due to assay-linked determinants.

All patients in our study, due to persistently elevated IGF-I, were given SSAs after neurosurgery. In our series, SSAs were able to control both GH and IGF-I in more than 50% of the patients, whereas as expected 11 patients showed persistently elevated GH and IGF-I levels (51). Interestingly, only one of these patients was d3-GHR, and this would seem in contrast with data recently reported suggesting that d3-GHR carriers may have a lower chance of IGF-I normalization with SSAs. However, interestingly, as after surgery, around 70% of patients with normal GH and high IGF-I were d3 carriers. The observation that almost 40% of patients carrying the d3-GHR allele showed discordant GH and IGF-I findings after SSAs is consistent with the hypothesis that lowering of GH levels after medical therapy may contribute further to the unmasking of GHR supersensitivity. These data suggest that particularly under medical treatment with SSAs, usefulness of binary criteria may be heavily challenged almost exclusively in patients carrying the d3-GHR isoform. Confirming this view, we also observed, as already reported in the literature (23), two patients that, under SSAs, showed normalized IGF-I with persistently elevated GH levels. This finding, which again could be interpreted in the light of GH assay variability, was associated with the fl-GHR, *i.e.* with presumed normal sensitivity to GH. Logistic regression analysis showed that the only predicting factor for biochemical discrepancy was the d3 allele. On the other hand, the d3 allele contributed independently to low GH levels after surgery together with presurgical GH levels. These findings confirm the clinical relevance of the determination of the d3 isoform in acromegaly, which can become one major predictor of biochemical treatment outcome of the disease. Moreover, they confirm the hypothesis of a modest value of GH assay after surgery and SSAs in d3-GHR carriers. In the current absence of known adjunctive factors able to predict discrepant behavior among d3 carriers, we suggest that based on our findings, evaluation of the d3 isoform could be part of the baseline characterization of the disease. We also propose very careful biochemical follow-up in d3 carriers, as well as, if our data will be confirmed, that normal ranges for IGF-I and particularly for GH should be obtained in d3-GHR acromegalic carriers.

In conclusion, we have described for the first time a clinically relevant impact of the polymorphism of the GHR on the post-treatment biochemical assessment of acromegaly. Increased sensitivity of the d3-GHR may be an independent predictor of GH and IGF-I discrepancy. Further studies are needed to characterize the GHR to find adjunctive characteristics strictly correlated with its supersensitivity and more strongly predictive in the d3 population of biochemical discrepancy.

## Acknowledgments

We are greatly indebted to Dr. Livio Fenga and Dr. Gherardo Mazziotti for performing and reviewing statistical analysis of our data.

Address all correspondence and requests for reprints to: Dr. Antonio Bianchi, Division of Endocrinology, Catholic University, School of Medicine, Largo A. Gemelli, 8, 00168 Rome, Italy. E-mail: abianchi69@yahoo.it.

Disclosure Summary: The authors have nothing to disclose.

## References

1. **Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI** 1987 Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature 330: 537–543
2. **Giustina A, Mazziotti G, Canalis E** 2008 Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev 29:535–559
3. **Kelly PA, Djiane J, Postel-Vinay MC, Edery M** 1991 The prolactin/growth hormone receptor family. Endocr Rev 12:235–251
4. **Godowski PJ, Leung DW, Meacham LR, Galgani JP, Hellmiss R, Keret R, Rotwein PS, Parks JS, Laron Z, Wood WI** 1989 Characterization of the human growth hormone receptor gene and demonstration of a partial gene deletion in two patients with Laron-type dwarfism. Proc Natl Acad Sci USA 86:8083–8087
5. **Pekhletsky RI, Chernov BK, Rubtsov PM** 1992 Variants of the 5′-untranslated sequence of human growth hormone receptor mRNA. Mol Cell Endocrinol 90:103–109
6. **Urbanek M, MacLeod JN, Cooke NE, Liebhaber SA** 1992 Expression of a human growth hormone (hGH) receptor isoform is predicted by tissue-specific alternative splicing of exon 3 of the hGH receptor gene transcript. Mol Endocrinol 6:279–287
7. **Wickelgren RB, Landin KL, Ohlsson C, Carlsson LM** 1995 Expression of exon 3-retaining and exon 3-excluding isoforms of the human growth hormone-receptor is regulated in an interindividual, rather than a tissue-specific, manner. J Clin Endocrinol Metab 80:2154–2157
8. **Dos Santos C, Essioux 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
9. **Binder G, Baur F, Schweizer R, Ranke MB** 2006 The d3-growth hormone (GH) receptor polymorphism is associated with increased responsiveness to GH in Turner syndrome and short small-for-gestational- age children. J Clin Endocrinol Metab 91:659–664
10. **Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ** 2006 Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab 91: 1076–1080
11. **Carrascosa A, Esteban C, Espadero R, Fernández-Cancio M, Andaluz P, Clemente M, Audí L, Wollmann H, Fryklund L, Parodi L** 2006 The d3/fl-growth hormone (GH) receptor polymorphism does not influence the effect of GH treatment (66 microg/kg per day) or the spontaneous growth in short non-GH-deficient small-for-gestational- age children: results from a two-year controlled prospective study in 170 Spanish patients. J Clin Endocrinol Metab 91:3281–3286
12. **Carrascosa A, Audí L, Fernández-Cancio M, Esteban C, Andaluz P, Vilaró E, Clemente M, Yeste D, Albisu MA, Gussinyé M** 2008 The exon 3-deleted/full-length growth hormone receptor polymorphism did not influence growth response to growth hormone therapy over two years in prepubertal short children born at term with adequate weight and length for gestational age. J Clin Endocrinol Metab 93:764–770
13. **Blum WF, Machinis K, Shavrikova EP, Keller A, Stobbe H, Pfaeffle RW, Amselem S** 2006 The growth response to growth hormone (GH) treatment in children with isolated GH deficiency is independent of the presence of the exon 3-minus isoform of the GH receptor. J Clin Endocrinol Metab 91:4171–4174
14. **Schmid C, Krayenbuehl 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
15. **Mercado M, González B, Sandoval C, Esquenazi Y, Mier F, Vargas G, de los Monteros AL, Sosa E** 2008 Clinical and biochemical impact of the d3 growth

hormone receptor haplotype in acromegaly. J Clin Endocrinol Metab 93:3411–3415
16. **Melmed S** 2006 Medical progress: acromegaly. N Engl J Med 355:2558–2573
17. **Kauppinen-Mäkelin R, Sane T, Reunanen A, Välimäki MJ, Niskanen L, Markkanen H, Löyttyniemi E, Ebeling T, Jaatinen P, Laine H, Nuutila P, Salmela P, Salmi J, Stenman UH, Viikari J, Voutilainen E** 2005 A nationwide survey of mortality in acromegaly. J Clin Endocrinol Metab 90:4081–4086
18. **Holdaway IM, Rajasoorya RC, Gamble GD** 2004 Factors influencing mortality in acromegaly. J Clin Endocrinol Metab 89:667–674
19. **Cazabat L, Souberbielle JC, Chanson P** 2008 Dynamic tests for the diagnosis and assessment of treatment efficacy in acromegaly. Pituitary 11:129–139
20. **Giustina A, Bresciani E, Bussi AR, Bollati A, Bonfanti C, Bugari G, Chiesa L, Giustina G** 1995 Characterization of the paradoxical growth hormone inhibitory effect of galanin in acromegaly. J Clin Endocrinol Metab 80:1333–1340
21. **Giustina A, Barkan A, Casanueva FF, Cavagnini F, Frohman L, Ho K, Veldhuis J, Wass J, Von Werder K, Melmed S** 2000 Criteria for cure of acromegaly: a consensus statement. J Clin Endocrinol Metab 85:526–529
22. **Mercado M, Espinosa de los Monteros AL, Sosa E, Cheng S, Mendoza V, Hernández I, Sandoval C, Guinto G, Molina M** 2004 Clinical-biochemical correlations in acromegaly at diagnosis and the real prevalence of biochemically discordant disease. Horm Res 62:293–299
23. **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
24. **Machado EO, Taboada GF, Neto LV, van Haute FR, Corrêa LL, Balarini GA, Shrank Y, Goulart M, Gadelha MR** 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
25. **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
26. **Bates AS, Evans AJ, Jones P, Clayton RN** 1995 Assessment of GH status in acromegaly using serum growth hormone, serum insulin-like growth factor-1 and urinary growth hormone excretion, Clin Endocrinol (Oxf) 42: 417–423
27. **Parfitt VJ, Flanagan D, Wood P, Leatherdale BA** 1998 Outpatient assessment of residual growth hormone secretion in treated acromegaly with overnight urinary growth hormone excretion, random serum growth hormone and insulin like growth factor-1. Clin Endocrinol (Oxf) 49:647–652
28. **Sheaves R** 1993 Interpretation of IGF-1 levels in the assessment of acromegaly. Current Medical Literature: Growth Hormone and Growth Factors 8:59–61
29. **Kaltsas GA, Isidori AM, Florakis D, Trainer PJ, Camacho-Hubner C, Afshar F, Sabin I, Jenkins JP, Chew SL, Monson JP, Besser GM, Grossman AB** 2001 Predictors of the outcome of surgical treatment in acromegaly and the value of the mean growth hormone day curve in assessing postoperative disease activity. J Clin Endocrinol Metab 86:1645–1652
30. **Boquete HR, Sobrado PG, Fideleff HL, Sequera AM, Giaccio AV, Suárez MG, Ruibal GF, Miras M** 2003 Evaluation of diagnostic accuracy of insulin-like growth factor (IGF)-I and IGF-binding protein-3 in growth hormone-deficient children and adults using ROC plot analysis. J Clin Endocrinol Metab 88: 4702–4708
31. **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
32. **Giustina A, Veldhuis JD** 1998 Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 19:717–797
33. **De Marinis L, Mancini A, Zuppi P, Anile C, Maira G** 1990 Paradoxical growth hormone response to thyrotropin-releasing hormone in acromegaly. Clinical correlations and prognostic value. Acta Endocrinol (Copenh) 122:443–449
34. **Giustina A, Bodini C, Doga M, Schettino M, Pizzocolo G, Giustina G** 1992 Galanin decreases circulating growth hormone levels in acromegaly. J Clin Endocrinol Metab 74:1296–1300
35. **Clemmons DR, Underwood LE** 1991 Nutritional regulation of IGF-I and IGF binding proteins. Annu Rev Nutr 11:393–412
36. **Thissen JP, Ketelslegers JM, Underwood LE** 1994 Nutritional regulation of the insulin-like growth factors. Endocr Rev 15:80–101
37. **Jones JI, Clemmons DR** 1995 Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 16:3–34
38. **Oppizzi G, Petroncini MM, Dallabonzana D, Cozzi R, Verde G, Chiodini PG, Liuzzi A** 1986 Relationship between somatomedin-C and growth hormone levels in acromegaly: basal and dynamic evaluation. J Clin Endocrinol Metab 63:1348–1353
39. **Lamberts SW, Uitterlinden P, Schuijff PC, Klijn JG** 1988 Therapy of acromegaly with sandostatin: the predictive value of an acute test, the value of serum somatomedin-C measurements in dose adjustment and the definition of a biochemical cure. Clin Endocrinol (Oxf) 29:411–420
40. **Hartman ML, Veldhuis JD, Vance ML, Faria AC, Furlanetto RW, Thorner MO** 1990 Somatotropin pulse frequency and basal concentrations are increased in acromegaly and are reduced by successful therapy. J Clin Endocrinol Metab 70:1375–1384
41. **Ho KY, Weissberger AJ** 1994 Characterization of 24-hour growth hormone secretion in acromegaly: implications for diagnosis and therapy. Clin Endocrinol (Oxf) 41:75–83
42. **van der Lely AJ, de Herder WW, Janssen JA, Lamberts SW** 1997 Acromegaly: the significance of serum total and free IGF-I and IGF-binding protein-3 in diagnosis. J Endocrinol 155(Suppl 1):S9–S13; discussion, S15–S16
43. **Barkan AL, Beitins IZ, Kelch RP** 1988 Plasma insulin-like growth factor-I/somatomedin-C in acromegaly: correlation with the degree of growth hormone hypersecretion. J Clin Endocrinol Metab 67:69–73
44. **Holly JM, Cotterill AM, Jemmott RC, Shears D, al-Othman S, Chard T, Wass JA** 1991 Inter-relations between growth hormone, insulin, insulin-like growth factor-I (IGF-I), IGF binding protein-1 (IGFBP-1) and sex hormone-binding globulin in acromegaly. Clin Endocrinol (Oxf) 34:275–280
45. **Rieu M, Girard F, Bricaire H, Binoux M** 1982 The importance of insulin-like growth factor (somatomedin) measurements in the diagnosis and surveillance of acromegaly. J Clin Endocrinol Metab 55:147–153
46. **Barreca A, Ciccarelli E, Minuto F, Bruzzi P, Giordano G, Camanni F** 1989 Insulin-like growth factor I and daily growth hormone profile in the assessment of active acromegaly. Acta Endocrinol (Copenh) 120:629–635
47. **Melmed S, Casanueva FF, Cavagnini F, Chanson P, Frohman L, Grossman A, Ho K, Kleinberg D, Lamberts S, Laws E, Lombardi G, Vance ML, Werder KV, Wass J, Giustina A** 2002 Acromegaly treatment consensus workshop participants. Guidelines for acromegaly treatment. J Clin Endocrinol Metab 87:4054–4058
48. **Melmed S, Casanueva F, Cavagnini F, Chanson P, Frohman LA, Gaillard R, Ghigo E, Ho K, Jaquet P, Kleinberg D, Lamberts S, Laws E, Lombardi G, Sheppard MC, Thorner M, Vance ML, Wass JA, Giustina A** 2005 Consensus statement: medical management of acromegaly. Eur J Endocrinol 153:737–740
49. **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
50. **Pokrajac A, Wark G, Ellis AR, Wear J, Wieringa GE, Trainer PJ** 2007 Variation in GH and IGF-I assays limits the applicability of international consensus criteria to local practice. Clin Endocrinol (Oxf) 67:65–70
51. **Murray RD, Melmed S** 2008 A critical analysis of clinically available somatostatin analog formulations for therapy of acromegaly. J Clin Endocrinol Metab 93:2957–2968