Analysis of *IGF(CA)19* and *IGFBP3-202A/C* gene polymorphisms in patients with acromegaly: association with clinical presentation and response to treatments

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Abstract

Objective: *IGF1* and *IGFBP3* gene polymorphisms have been recently described. However, their potential role in the setting of acromegaly and its outcome is unknown. In this study, we analyze these polymorphisms in patients with acromegaly and investigate their association with clinical presentation and response to treatments.

Design: A retrospective observational study was conducted in patients with acromegaly to analyze *IGF1* and *IGFBP3* gene polymorphisms.

Methods: A total of 124 patients with acromegaly (57.3% women, mean age 44.9 ± 13.1 years old) were followed up for a period of 11.4 ± 8.0 years in eight tertiary referral hospitals in Spain. Clinical and analytical data were evaluated at baseline and after treatment. *IGF1* and *IGFBP3* gene polymorphisms were analyzed using PCR and specific primers.

Results: Baseline laboratory test results were GH 19.3 (8.0–39.6) ng/ml, nadir GH 11.8 (4.1–21.5) ng/ml, and index IGF1 2.65 ± 1.25 upper limit of normal. Regarding the *IGF1* gene polymorphism, we did not find any association between the number of cyto-adenosine (CA) repeats and patients' baseline characteristics. Nevertheless, a trend for higher nadir GH values was observed in patients with < 19 CA repeats. Regarding the *IGFBP3* polymorphism, the absence of an A allele at the –202 position was associated with a higher baseline IGF1 and a higher prevalence of cancer and polyyps. There were no differences in response to therapies according to the specific genotypes.

Conclusions: Polymorphisms in the *IGF1* and *IGFBP3* genes may not be invariably determinant of treatment outcome in acromegalic patients, but they may be associated with higher nadir GH levels or baseline IGF1, and determine a higher rate of colorectal polyps and cancer.

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Introduction

Acromegaly is a rare disease that occurs due to an endogenous excess of growth hormone (GH) and insulin-like growth factor 1 (IGF1) levels, which entails acral enlargement, metabolic abnormalities, and an increased risk of morbidity and mortality (1).

GH secretion leads to IGF1 synthesis in the liver and several peripheral tissues. Around 99% of circulating IGF1 binds to IGF-specific binding proteins (IGFBPs), which modify its bioavailability by extending its half-life and modulating its biological actions on target tissues. IGFBP3 is the most abundant circulating subtype, which transports more than 75% of serum IGFs (2).

Polymorphisms in the promoter regions of the *IGF1* and *IGFBP3* genes, which are encoded in chromosomes 12q and 7p respectively, have been recently described. These polymorphisms could hypothetically modify the clinical expression and the response to treatments in acromegaly.

The *IGF1* gene polymorphism consists of a highly polymorphic microsatellite composed of a variable number of cyto-adenosine (CA) repeats (from 10 to 24), situated 1 kb upstream of the transcription site of IGF1. The most common allele in the Caucasian population contains 19 CA repeats (192 bp) (3, 4). Differences in the number of CA repeats have been associated with serum IGF1 levels, although controversial results have been communicated (3, 5, 6, 7, 8). In addition, the number of CA repeats has been associated with various clinical conditions or risk situations, such as age-related IGF1 decline (7), final adult height (4, 5, 9, 10), risk of developing diabetes mellitus and myocardial infarction (5, 11), survival after a myocardial infarction in diabetic patients (12), efficacy of recombinant human GH (rhGH) treatment in GH-deficient children, and Turner's syndrome (13, 14, 15) and cancer susceptibility (16).

Regarding the *IGFBP3* gene, recent research has revealed the presence of five single-nucleotide polymorphisms (SNPs) in the *IGFBP3* promoter region, of which the most relevant was an A to C nucleotide change located 202 bp upstream of the transcription site (–202A/C). The promoter activity is significantly higher in A allele carriers;

in other words, IGFBP3 circulating levels have been reported to be correlated with the number of A alleles at this site, being higher in AA, and decreasing in a stepwise manner in AC and CC (17). Data regarding the distribution of this polymorphism in the general population and in different clinical settings are heterogeneous and somehow confusing (14, 18, 19, 20).

In the particular case of acromegaly, the role and clinical implications of both polymorphisms have not been fully explored. In this study, we aimed to analyze i) the prevalence of the different genotypes in the promoter regions of the *IGF1* and *IGFBP3* genes (number of CA repeats and –202A/C respectively) in patients with acromegaly and ii) their impact on the severity of acromegaly (clinical, biochemical, and comorbidities) and its outcome.

Subjects and methods

Study population

We performed a retrospective observational study of 124 (71 women, 57.3%) unrelated patients who were diagnosed with acromegaly according to current guidelines (1), from eight tertiary care referral hospitals in Spain. All patients had GH-secreting pituitary adenomas. The ethics committees of each hospital approved the protocol, and all patients signed a written informed consent before inclusion. Some patients included in this study have also been previously evaluated in other studies (21, 22).

Data regarding physical examination, medical history, and laboratory work-up were obtained from routine visits using information available in clinical records. The following evaluations were collected at diagnosis (baseline): weight and height, and the corresponding BMI (kg/m^2), pituitary imaging studies, liver tests, basal and nadir GH levels, IGF1 levels expressed in reference to the upper limit of normal (ULN), and associated comorbidities. Although this was a non-interventional study and each treating physician managed patients at their discretion, general recommendations were followed. In this regard,

when feasible, surgery was the first treatment of choice. If surgical cure was not achieved, medical therapy (monotherapy or combination) and/or radiotherapy were implemented, depending on the specific case. Follow-up evaluations included pituitary magnetic imaging, laboratory tests, registry of adverse effects to treatment, and verification of disease control and/or cure.

Assessment of comorbidities

At diagnosis, patients were evaluated for the presence of complications and co-existing illnesses, including dyslipidemia, diabetes, abnormal liver function tests (LFTs), sleep apnea, cardiomyopathy, cerebrovascular disease, arthropathy and nerve entrapment (carpal tunnel syndrome), presence of colon polyps, goiter, and cancer, as currently recommended (23).

Biochemical and hormonal assays

After an overnight fast, blood samples were collected from patients at baseline and different follow-up times. Random and nadir GH and serum IGF1 levels were measured in hospital laboratories and interpreted according to the local age- and sex-based reference ranges. Results were then given as the total IGF1 level, and in order to generate a standard value that could be compared among centers, it was expressed as X-fold the individual ULN. Measurement of IGF1 was based on immunochemiluminescent (Immulite GH; EURO/DPC, Gwynedd, UK) and immunoradiometry (Immunotech–Beckman, Marseille, France and Diagnostic Systems, Inc., Upper Heyford, Oxon, UK) methods. GH was analyzed using immunochemiluminescence (Immulite GH; DPC, Los Angeles, CA, USA) and immunoradiometry (DiaSorin, Vercelli, Italy).

Molecular studies

Genomic DNA was isolated from peripheral blood leucocytes from the 124 acromegalic patients and analyzed to determine the *IGF(CA)* repeats and *IGFBP3-202A/C* polymorphisms, using specific primers by PCR methods, as described previously (24). All molecular studies were centrally performed.

Statistical analysis

Descriptive results were expressed as mean $\pm$ s.d. and median (interquartile range) for normally and non-normally distributed continuous variables respectively.

Categorical variables were summarized as frequencies and percentages. For the purpose of this study, and although there is no specific consensus on how to study these polymorphisms, we grouped them into different categories, as suggested by others (5, 6, 9, 19, 20, 24), and analyses were performed using all classifications. Possible existing associations of baseline characteristics with genotype variants were evaluated using two-sided ANOVA. Comparison between categorical groups was assessed by the χ^2 test (Fisher's exact test as required). The *P* values were two-sided and statistical significance was considered when *P* < 0.05. All statistical analyses were performed using SPSS version 19.0 (IBM SPSS Statistics, Inc.).

Results

A total of 124 acromegalic patients (71 women, 57.3%), with a mean age of 44.9 ± 13.1 years, were evaluated and followed for a mean period of 11.4 ± 8.0 years. Patients' baseline clinical characteristics were: BMI 28.2 ± 5.0 kg/m², basal GH 19.3 (8.0–39.6) ng/ml, post-oral glucose tolerance test (OGTT) nadir GH 11.8 (4.1–21.5) ng/ml, index IGF1 ($\times$ ULN) 2.65 ± 1.25 , and tumor diameter 19.0 (12.5–25.5) mm. We observed the following associated comorbidities: 46 patients (37.1%) had dyslipidemia, 49 (39.5%) had diabetes, nine (7.3%) presented abnormal LFTs, 12 (9.7%) suffered obstructive sleep apnea, 19 (15.3%) had cardiomyopathy, six (4.8%) had cerebrovascular disease, 19 (15.3%) exhibited arthropathy (nerve entrapment), 25 (20.2%) had colonic polyps, 41 (33.1%) with goiter, and 14 patients (11.3%) had cancer. Pituitary surgery was performed in a total of 63 patients (50.8%) as primary therapy. Primary medical therapy with somatostatin analogs was started in 55 patients (44.4%) because of surgical contraindications and/or preference for medical management, and in 86 cases (69.3%) they were used as adjuvant therapy. The remaining six patients (4.2%) were followed conservatively. Thirty-five patients (28.2%) required switching to pegvisomant treatment as monotherapy (30 subjects), or combination treatment (five individuals) because of insufficient control under somatostatin analog treatment. Radiotherapy was performed in 44 patients (35.5%). Overall, control of GH and IGF1 levels was achieved in 70.2% of patients, and mean time from diagnosis to control of IGF1 levels was 5.8 ± 5.9 years.

Complete clinical and demographic data were registered in all subjects. Genotypes of the IGF1 promoter region polymorphisms were available in 113 patients (Fig. 1), and we observed that the genotype frequencies were in Hardy–Weinberg equilibrium. For statistical

Figure 1

Number (percentage) of patients in each polymorphism group. (A) *IGF(CA)* genotypes according to the number of bps (CA repeats): <192 bp (<19 CA repeats); 192/194 bp (19/20 CA repeats); and >194 bp (>20 CA repeats). (B) *IGF(CA)* genotypes according to the presence or absence of the WT allele *IGF(CA)19* (192/192 bp). (C) *IGFBP3* polymorphism, grouped as CC/CA/AA genotypes. (D) *IGFBP3* polymorphism grouped as AA vs non-AA.

purposes, *IGF(CA)* genotypes were grouped according to the presence or absence of the WT allele *IGF(CA)19* (192/192 bp), and also according to the presence of <192 bp (<19 CA repeats), 192/194 bp (19/20 CA repeats) and >194 bp (>20 CA repeats). Figure 1A and B shows the distribution of this polymorphism in our patients.

We did not find any significant differences between groups regarding clinical presentation and comorbidities. There was no association between *IGF(CA)* genotype and baseline GH and IGF1 concentrations. However, we found a trend ($P=0.064$) for higher post-OGTT nadir GH in patients with a lower number of CA repeats. Nadir post-OGTT GH value was 17.8 (13.3–31.1) ng/ml in <192 bp (<19 CA repeats); 11.7 (3.4–25.0) mg/ml in the 192–194 bp group (19 and 20 CA repeats) and 5.5 (4.0–19.0) ng/ml in those with >194 (>20 CA repeats) (Fig. 2A). Additionally, nadir GH levels were higher in patients who did not carry the WT allele (192/192 bp) for the *IGF(CA)19* polymorphism (6.8 (2.5–19.4) ng/ml vs 15.6 (6.3–22.2) ng/ml, Fig. 2B). Response to treatments, the frequency of secondary adverse outcomes, and the number of patients who achieved biochemical control were not significantly different between the various *IGF(CA)19* genotype groups.

The *IGFBP3-202A/C* polymorphism was studied in 119 patients. In this case, genotype distributions did not follow the Hardy–Weinberg equilibrium, mainly due to a defect in heterozygosis, which could be partially explained by the Wahlund effect, i.e. a mixture of populations. Patients were grouped as CC, CA, and AA genotypes, and also as AA vs non-AA group. The frequency of its distribution is shown in Fig. 1C and D. Baseline index IGF1 was different among the three allele groups: $3.1 \pm 1.5\%$ in the CC genotype; $2.1 \pm 1.0\%$ in the AC genotype; and $2.7 \pm 1.1\%$ in the AA genotype, $P=0.025$. Pairwise comparison showed differences in index IGF1 between CC and AC ($P=0.017$) (Fig. 3A). However, there was no significant difference in baseline index IGF1 between carriers and non-carriers of the WT allele (AA) (Fig. 4B). Interestingly, colonic polyps ($P=0.027$) and cancer ($P=0.019$) were more frequent in those patients who carried the CC allele (Fig. 4A). In fact, when groups were compared according to the presence or absence of the WT allele (AA), colonic polyps were more frequent among the non-carriers (Fig. 4B). Additionally, we could observe that patients with the AC genotype required a longer period of time to achieve control of IGF1 levels (3.9 ± 6.2 years in CC, 7.6 ± 6.5 years in AC, and 5.2 ± 4.0 years in AA, $P=0.049$), although no significant differences were obtained when comparing the WT and non-WT groups (5.8 ± 6.5 years in non-AA and 5.1 ± 4.0 years in AA, $P>0.05$). No other influences in the response to treatments, the number of patients who achieved biochemical control, or the frequency of secondary adverse outcomes was observed.

Figure 2

Post-oral glucose tolerance test (OGTT) nadir GH values, according to the *IGF(CA)* genotype. (A) Post-OGTT nadir GH values were 17.8 (13.3–31.1) ng/ml in <192 bp (<19 CA repeats); 11.7 (3.4–25.0) mg/ml in 192–194 (19 and 20 CA repeats); and 5.5 (4.0–19.0) ng/ml in >194 (>20 CA repeats). (B) In non-carriers of the WT allele (192/192 bp), post-OGTT nadir GH levels were 6.8 (2.5–19.4) ng/ml vs 15.6 (6.3–22.2) ng/ml in WT carriers. P values are shown for ANOVA (the Kruskal–Wallis or Mann–Whitney U test, accordingly).

Figure 3

Baseline index IGF1 (referred to the upper limit of normal – $\times$ ULN) according to the *IGFBP3* genotype groups. (A) Baseline index IGF1 according to CC/AC/AA allele groups. *P* value for comparison among the three groups was $P=0.025$ ($3.1 \pm 1.5\%$ in CC genotype; $2.1 \pm 1.0\%$ in AC genotype; and $2.7 \pm 1.1\%$ in AA genotype). Pairwise comparison showed differences in IGF1 index levels between CC and AC. (B) Baseline index IGF1 according to non-AA or AA genotypes. No significant differences in IGF1 baseline levels were observed between carriers and non-carriers of the WT allele (AA).

Discussion

In this study, we analyzed the *IGF(CA)* and *IGFBP3-202A/C* genotypes in 113 and 119 acromegalic patients, respectively, and evaluated their impact on clinical and biochemical expression of acromegaly and its outcome. To our knowledge, this is the largest cohort of acromegalic patients in whom these polymorphisms have been evaluated, as only a previous study in 34 acromegalic patients has been reported (24).

The majority of our patients (73%) carried the 192 bp variant (19 CA repeats) of the *IGF(CA)* polymorphism, similar to what has been reported in a normal population-based sample (5).

The potential functional relationship between the *IGF(CA)* polymorphism and circulating IGF1 levels has been addressed in several publications, with conflicting results. Earlier studies performed in a large sample of the Dutch population from the Rotterdam Study (5, 6, 7) found higher IGF1 concentrations in carriers of 192 or 194 bp alleles (19 or 20 CA repeats). However, subsequent studies did not corroborate these findings in the UK population (10), or even found the opposite, such as a study conducted in men with idiopathic osteoporosis, in which lower serum IGF1 levels were observed in carriers of 192/192 bp (3). Furthermore, in adult patients with GH deficiency, the length of the CA microsatellite was not associated with an increased IGF1 response to rhGH

treatment (25). Finally, in a small series of acromegalic patients, higher IGF1 levels and a more severe disease was found in those patients who carried the >194 bp (20 CA repeats or greater) variant (24).

In our study, we did not find any significant association between baseline IGF1 levels and any specific *IGF(CA)* genotype. The underlying reasons for the discrepancies observed with previous studies may include difficulties and variability in measurements of serum IGF1 concentrations, heterogeneity of populations studied (i.e. healthy controls, GH-deficient individuals, and acromegalic patients), as well as the possible existence of other regulatory elements of the *IGF1* gene. In this regard, in our study, IGF1 levels were referred to the ULN for age and sex, in order to bypass incongruities between laboratory methods. Also, all of our patients had been diagnosed with acromegaly. This may also explain the absence of an association between the number of CA repeats and IGF1 levels, in comparison with healthy controls or GH-deficient individuals, as the GH/IGF1 axis in acromegaly behaves in a rather aberrant way. We did not confirm the finding of a more severe disease in cases with the >194 bp variant reported previously (24) in a smaller sample of acromegalic patients (34 cases). Moreover, it must be noted that the CA microsatellite is located at a region that contains specific regulatory elements of the *IGF1* gene. Consequently, some authors have speculated that allelic variation in this region might lead to changes in the promoter activity or might be in linkage disequilibrium with another sequence in the promoter region, leading to an alteration in IGF1 transcription and

Figure 4

Percentage of patients with colonic polyps (dark gray bars) and cancer (light gray bars). (A) According to CC/AC/AA *IGFBP3* genotypes and (B) according to non-AA or AA genotypes. *P* values for χ^2 analysis comparing the three groups were $P=0.027$ for colonic polyps and $P=0.019$ for cancer. *P* values for comparison between pairs are shown in the figure.

subsequent IGF1 circulating levels (26). In view of these findings, we can point out that the precise role of the *IGF(CA)* polymorphism in IGF1 levels in general population remains yet to be elucidated. However, our data suggest that, in acromegalic patients, the length of the CA microsatellite in the *IGF1* gene promoter region is not related to serum IGF1 levels.

There are no previous reports on the relationship between this *IGF(CA)* polymorphism and GH levels in acromegalic patients or healthy controls. In this study, we found higher post-OGTT nadir GH levels in patients who carried a lower number of CA repeats, as well as in non-carriers of the WT allele. Reasons explaining these findings deserve further discussion. A possible explanation may be that an increased number of CA repeats could be associated with a higher resistance to physiological GH suppression after an OGTT. In fact, although we did not find any differences in genotypes according to sex, this has been observed in women, who usually exhibit higher GH nadir values after an OGTT (27). Therefore, it could be hypothesized that the *IGF(CA)* polymorphism could also influence GH nadir values, in a similar way to what is observed with sex.

Regarding the *IGFBP3* genotype, several studies performed in healthy controls, adult GH-deficient patients, children born small for gestational age, and in patients with cancer (9, 13, 17, 18, 28) showed a higher promoter activity (and higher IGFBP3 circulating levels) in AA > AC > CC genotypes.

In our study, the distribution of the three genetic variants was 25.2% CC, 30.2% AC, and 44.5% AA, which is the WT allele. Reports concerning the IGFBP3 polymorphism in the setting of acromegaly are scarce, and, to our knowledge, only a study reporting 25 acromegalic patients has been published (24). In this small series, these different genotypes were not associated with any clinical or biochemical characteristics, nor with the outcome or adverse events of treatments. By contrast, in our study, baseline index IGF1 was significantly different between the three genotype variants ($3.1 \pm 1.5\%$ in CC, $2.1 \pm 1.0\%$ in AC, and $2.7 \pm 1.1\%$ in AA). Hypothetically, the AA genotype would determine higher IGFBP3 levels and, consequently, a lower bioavailable free IGF1 and a higher stimulation of GH secretion by the feedback mechanism of the somatotrophic axis, which is usually preserved in patients with somatotropinomas (22). Unfortunately, in our study, we did not measure IGFBP3 levels, because, unlike in the follow-up of GH-deficient individuals, this is not a routine practice in the management of acromegaly. The correlation between the IGFBP3 polymorphism

and serum IGF1 levels has not been observed in other studies (24, 28, 29) that evaluated different populations or a small cohort of acromegalic patients.

Regarding acromegaly-associated comorbidities, some reports have suggested that non-carriers of the 192 bp homozygous allele might have an increased risk of developing type 2 diabetes and myocardial infarction (5). However, this was not the case in our study, probably due to the high prevalence of individuals with the 192–194 bp and WT variants. However, we found an increased number of patients with colonic polyps and cancer among those with the *IGFBP3* CC genotype. Given the relationship between increased circulating levels of IGF1 and IGF1/IGFBP3 molar ratio and colorectal adenomatous polyps (30), our finding is probably related to the fact that the CC group exhibited the highest baseline IGF1 index levels. To the best of our knowledge, no previous studies have been carried out analyzing the 202A/C polymorphism in patients with colonic adenomas or carcinomas. Nevertheless, a previous study carried out in non-acromegalic patients (31) analyzed several candidate polymorphisms in selected genes of *IGF1*, *IGF1R*, *IGFBP3*, and *GH1* in the development of colorectal adenomas. Although they did not find any differences in the +227 C/G polymorphism of the *IGFBP3* gene, their results indicated that subjects who carried a single copy of the A allele in the IGF1-704 T/C polymorphism were at a reduced risk for colorectal adenomas. Further research should be carried out to confirm these results.

The possible implication of gene polymorphisms in the onset of acromegaly, the efficacy and safety of available therapies, and the final outcome has been a matter of great interest over the past recent years (21, 22, 32, 33). Regarding the particular case of *IGF(CA)* and *IGFBP3*-202A/C polymorphisms, data are still scarce. Only a small study (24) found that patients having the > 194 bp *IGF(CA)* genotype had a more active disease and required higher doses of medication or combination therapies, although they did not report further details explaining this issue.

Our results did not portray differences in response to treatments or adverse effects according to the genetic variant of either polymorphism, suggesting that genotype variants of the *IGF(CA)* and IGFBP3 polymorphisms may have only a minor influence in treatment outcome. This is biologically plausible, as current treatments for acromegaly mainly act via the SSTRs and GHR pathways. However, *IGF(CA)* and IGFBP3 polymorphisms may be associated with higher nadir GH levels and baseline index IGF1, respectively, which may determine a higher rate of colorectal polyps and cancer.

Our study has some limitations. First, strictly speaking, a genetic analysis to assess the possible relationship between genotype and phenotype would require a much larger and homogeneous sample of patients. In this regard, the required sample size for a pharmacogenetic study would never be reached in a low prevalence disease such as acromegaly. Although we studied a considerably large number of patients, we acknowledge that the impact of a single study may be somehow insufficient to draw definitive pharmacogenetic conclusions. Another consideration to be taken into account is that even though management of acromegaly is generally standardized over our country, and the follow-up period of our cohort was more than 10 years, the retrospective nature of our study conveys certain limitations in the interpretation of these results. However, we consider that our findings entail a relevant clinical significance, as knowledge of the patients' specific polymorphic genotype may help predict specific phenotypic associations. Further larger and long-term prospective studies are necessary to verify whether these polymorphisms influence the severity of acromegaly and response to treatments.

Declaration of interest

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Author contribution statement

A M Ramos-Leví researched, analyzed, and interpreted data and wrote the manuscript. M Marazuela and I Bernabeu contributed to study conception and design, followed-up patients, interpreted data, and reviewed and edited the manuscript. A Paniagua, J Riveiro, C Álvarez-Escolá, T Lucas, C Blanco, P de Miguel, P M de Icaya, and I Pavón researched data and followed-up patients. C Quinteiro performed all molecular studies. All authors contributed to the final version of this manuscript.

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