CLINICAL STUDY

D3 GH receptor polymorphism is not associated with IGF1 levels in untreated acromegaly

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Abstract

Context: A discrepancy between serum GH and IGF1 concentrations is frequent in patients with acromegaly. Here, we examined whether the exon 3-deleted (d3) GH receptor (*GHR*) variant, which has been linked to increased responsiveness to GH treatment in short children, influences the GH/IGF1 relationship in patients with acromegaly.

Objective: To study the possible influence of the *GHR* genotype on the GH/IGF1 relationship in untreated acromegalic patients.

Design: *GHR* genotype analysis with retrospective clinical and biochemical data collection performed in a single third-reference medical center.

Patients and methods: Clinical data were obtained from the medical records of 105 acromegalic patients who had GH and IGF1 assays in the same laboratory and who were genotyped for the full-length (fl) or d3-*GHR* alleles.

Results: The distribution of *GHR* genotypes was 51% fl/fl, 30% fl/d3, and 19% d3/d3. Patients with d3/d3 genotype were younger than the patients in the other two groups ($P < 0.05$). Baseline GH and IGF1 concentrations did not differ among the three groups. The linear correlation between GH and IGF1 concentrations was similar in the three genotypic groups.

Conclusions: The exon 3 *GHR* genotype does not affect the GH/IGF1 relationship in untreated acromegalic patients with high circulating GH and IGF1 levels.

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Introduction

Randomly measured serum GH and insulin-like growth factor-1 (IGF1) concentrations are currently used as indices of acromegaly disease activity (1). The relationship between $\log_{10}$ serum GH and serum IGF1 concentrations is linear in patients with active disease (2, 3), but discordant serum GH and IGF1 levels are observed in many acromegalic patients (2, 4–6). Although the serum IGF1 level is mainly dependent on the GH level in acromegaly, IGF1 responsiveness to GH may be modulated by other factors, including gender, age, nutrition, body composition, and previous therapy (2, 7, 8). Genetic factors may also influence individual GH sensitivity. The GH receptor gene (*GHR*) is an obvious candidate. Among other polymorphisms, *GHR* bears a common microdeletion leading to exon 3

retention (full-length *GHR*, fl) or exclusion (exon 3-deleted *GHR*, d3) (9). Approximately half of Europeans carry at least one d3 allele (10). We have previously observed a link between the d3-*GHR* variant and increased responsiveness to GH therapy in short children, and demonstrated increased GH signal transduction by the transfected d3-*GHR* isoform (11). Subsequent studies extended this observation to children with Turner's syndrome (12), children with GH deficiency (GHD) (13, 14), and adults with GHD (15). However, for reasons that are controversial (16), other teams failed to confirm these findings in GHD (17, 18) or short for gestational age children (19, 20). The influence of the *GHR* genotype on human postnatal growth velocity and final adult height is also a matter of debate (21, 22). Finally, two recent studies have analyzed the impact of d3-*GHR* polymorphism in

acromegalic patients leading, once again, to divergent conclusions (10, 23). Here, we analyzed the influence of the exon 3 *GHR* genotype on the GH/IGF1 relationship in a cohort of 105 untreated acromegalic patients.

Patients and methods

Study population

We reviewed data on 105 acromegalic patients (57 women and 48 men). Acromegaly was diagnosed between January 1993 and February 2007, based on the usual criteria (1). Clinical parameters (age, gender, height, weight, pituitary tumor size, anterior pituitary insufficiency and hormone replacement therapy, glucose intolerance or diabetes mellitus, and arterial hypertension at diagnosis) and biological data (mean serum GH and IGF1 concentrations prior to acromegaly treatment) were collected from the medical records. Median age at diagnosis was 43 years (range 20–75 years). Eighty-three patients (79%) presented with a pituitary macroadenoma and 22 (21%) patients with a pituitary microadenoma. Forty-four patients (42%) had anterior pituitary hormone deficits, nine of them had panhypopituitarism. Four patients were receiving oral estrogen, two transdermal estrogen, and one androgen replacement therapy. Impaired glucose tolerance or diabetes mellitus was present in 39 (37%) patients and arterial hypertension in 35 (33%) patients. A blood sample was taken from each patient for *GHR* genotyping between January 2003 and February 2007.

Hormone measurements

Serum GH was measured with highly sensitive solid-phase two-site sandwich assays. As several assays were used, we converted GH concentrations from ng/ml to mIU/l by using the appropriate conversion factors for each method and standard. Reported basal GH serum concentrations are the mean values for at least four serum samples obtained during an hourly profile. Serum IGF1 concentrations were measured as described by Mercado *et al.* (24). Briefly, until 1998, sera were gel filtered on Ultrogel AcA 54 columns in acetic acid. RIA was used for IGF1, with a specific polyclonal antihuman IGF1 antibody. Recombinant human IGF1 was used as both standard and tracer. Unknown samples were studied at three concentrations, each in duplicate, plus one blank (tube without antibody). Intra- and inter-assay coefficient of variations were 4.8 and 10% respectively. After 1998, the same IGF1 RIA was used after separating IGF from binding proteins, in a new method. Plasma was incubated for 30 min at room temperature in acidic medium (0.01 M HCl) and then ultrafiltered on a Centricon 30. After lyophilization, the IGF-containing ultrafiltrate was taken up in 0.1 M phosphate buffer (0.1% BSA, pH 7.4) and analyzed by

RIA. Importantly, the reference IGF1 concentrations with this method were the same as with the previous method. Thus, throughout this retrospective study, reference IGF1 concentrations in our laboratory remained unchanged: normal-for-age serum levels of IGF1 (mean $\pm$ s.d. in μ g/l) were as follows: 16–20 y, 405 ± 70 ; 20–30 y, 310 ± 55 ; 30–40 y, 275 ± 50 ; 40–50 y, 245 ± 50 ; 50–60 y, 215 ± 50 ; 60–70 y, 185 ± 50 ; 70–80 y, 165 ± 50 ; and > 80 y, 155 ± 50 . Age-adjusted IGF1 values were calculated with age-specific reference ranges for our IGF1 assay ($\text{IGF1\%} = \text{patient's IGF1/age-specific upper limit} \times 100$; the age-specific upper limit = mean IGF1 normal for age and sex $+ 2$ s.d.). We did not use individual IGF1 SDS for two reasons: first, it is well known that the distribution is not Gaussian in healthy adults, necessitating correction to obtain the SDS; and second, many IGF1 values exceeded five, at which SDS is considered irrelevant at the individual level.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes. The *GHR* exon 3 genotype (fl/fl, d3/fl, and d3/d3) was determined by simple multiplex PCR as described by dos Santos *et al.* (11) and confirmed by allele-specific PCR (25).

Ethics

Written informed consent for genetic analysis was obtained from each patient. The study was approved by the local ethics committee and was performed in keeping with French legislation.

Statistical analysis

Data are reported as means $\pm$ s.e.m. For correlation analyses, serum GH concentrations were expressed as $\log_{10}$ values. Data were analyzed with Prism version 4 and statistical analysis software (SAS). The level of significance was set at $P = 0.05$. Groups were compared, with χ^2 -test, Fischer's exact test, Kruskal–Wallis test followed by Dunn's multiple comparison test, Wilcoxon rank test, and Spearman rank correlation analysis. To evaluate the influence of the *GHR* genotype on the baseline serum IGF1 concentration, we fitted a multiple linear regression model with age, gender, body mass index (BMI), and serum GH concentrations as potentially predictive variables.

Results

The *GHR* genotypes were fl/fl in 54 cases (51%), d3/fl in 31 cases (30%), and d3/d3 in 20 cases (19%). Baseline clinical and biochemical characteristics of the three genotypic groups are shown in Table 1. There was no

Table 1 Baseline clinical and biological characteristics of the acromegalic patients, according to the GH receptor (*GHR*) genotype.

Genotype	fl/fl	fl/d3	d3/d3	P
n	54	31	20	
Age (years)	46 ± 2	44.5 ± 2.4	35.7 ± 2.3	<0.05
Sex (F/M)	33/21	13/18	11/9	NS
Height (cm)	171.0 ± 1.4	172.7 ± 1.5	173.7 ± 2.5	NS
BMI	26.5 ± 0.6	27.5 ± 0.6	26.2 ± 0.8	NS
Macroadenoma (%)	81	77	75	NS
Pituitary deficiency (%)	43	52	25	NS
Diabetes/IGT (%)	37	39	35	NS
Hypertension (%)	33	39	25	NS
GH (mIU/l)	63.7 ± 10.1	74.7 ± 20.3	93.8 ± 26.4	NS
IGF1 (ng/ml)	992.4 ± 52.4	957.0 ± 75.6	1029.0 ± 95.7	NS
IGF1 (%)	289.2 ± 16.1	289.2 ± 28.4	284.3 ± 25.4	NS

Values are means ± S.E.M.; IGF1 (%) is the percentage of the upper limit (mean IGF1 + 2 S.D.) of the age-specific references range of IGF1. IGT, impaired glucose tolerance.

difference in gender, height, BMI, percentage of macroadenomas, frequency of impaired glucose tolerance/diabetes mellitus, frequency of arterial hypertension, or the pre-treatment GH and IGF1 concentrations across the three groups, but d3/d3 patients were younger than the patients in the other two groups. As expected, a linear correlation between serum GH and IGF1 concentrations was observed in the three groups (Fig. 1) according to closely similar equations. Similar linear associations were found when IGF1 values were adjusted for age. The multiple regression model showed that IGF1 concentrations were correlated with $\log_{10}$ serum GH ($P < 0.0001$), seemed to be associated with gender without reaching statistical significance ($P = 0.07$), and did not correlate with age, BMI, or the *GHR* genotype.

Discussion

As GH and IGF1 concentrations are used as indices of disease activity in acromegaly (1), factors potentially influencing the GH/IGF1 axis are of major clinical importance. Here, we analyzed whether the d3-*GHR* variant, which has been linked to increased responsiveness to GH treatment in short children, influences the GH/IGF1 relationship in patients with untreated acromegaly.

Two recent studies have previously examined the impact of *GHR* variants on serum GH and IGF1 concentrations in acromegalic patients. Schmid *et al.* reported, in a cohort of 44 untreated acromegalic patients, lower GH concentrations in d3-*GHR* carriers than in fl/fl patients, whereas the IGF1 concentrations did not differ among the *GHR* genotype groups. The authors suggested that patients carrying d3-*GHR* allele require lower GH concentrations to produce a given increase in serum IGF1 and to develop acromegalic symptoms (10). By contrast, Mercado *et al.* found no difference in GH or IGF1 concentrations in 152 acromegalic patients at diagnosis, but d3-*GHR*-carrying

patients presented with higher IGF1 concentrations after acromegaly treatment. Interestingly, the exon 3 deletion was a stronger predictor of persistently elevated IGF1 concentrations during post-treatment follow-up than age at diagnosis, gender, and even baseline GH and IGF1 levels or tumor size. Surprisingly, the expected correlation between IGF1 and $\log_{10}$ GH concentrations was only weak in d3-*GHR* patients and was not confirmed in fl-*GHR* patients (23).

In our study, which involved 105 untreated acromegalic patients, the distribution of the *GHR* genotypes was comparable with previous study populations (10, 11, 23). Of note, d3/d3 carriers were significantly younger at diagnosis than fl/fl and fl/d3 patients, suggesting that d3-*GHR* could promote the occurrence of acromegalic phenotype at a younger age. Nevertheless, such difference was not found in previous studies (10, 23). At variance with the findings of

Figure 1 Association between baseline IGF1 concentrations and $\log_{10}$ GH concentrations in acromegalic patients with full-length *GHR* (fl/fl) and exon 3-deleted *GHR* variants (d3/fl and d3/d3) at diagnosis. No influence of the *GHR* genotype was found (fl/fl: $\text{IGF1} = 377 \log_{10} \text{GH} + 411$, $R = 0.50$, $P < 0.0001$; d3/fl: $\text{IGF1} = 365 \log_{10} \text{GH} + 391$, $R = 0.53$, $P < 0.001$; d3/d3: $\text{IGF1} = 346 \log_{10} \text{GH} + 448$, $R = 0.46$, $P < 0.05$).

Schmid *et al.* (10) and in accordance with the recent study of Mercado *et al.* (23), we observed no difference in baseline GH and IGF1 concentrations across the *GHR* genotypic groups at the time of acromegaly diagnosis. This negative finding is corroborated in our study by clear linear association between serum IGF1 and log₁₀ GH according to closely similar equations among the *GHR* genotypes.

In vitro, GH signal transduction by transfected d3-GHR isoforms is increased when using both physiological and supraphysiological GH concentrations, similar to those observed in active acromegalic patients (11). Nevertheless, chronic exposition to such grossly excessive GH concentrations *in vivo* may mask the enhanced GH sensitivity of d3-GHR. Considering the data of Mercado *et al.* d3-GHR genotype may possibly influence the GH/IGF1 relationship only when patients have recovered more normal circulating GH concentrations after acromegaly treatment. When GH concentrations are very high, owing to unrestricted chronic tumoral secretion, the influence of the *GHR* variant (if any) is undetectable, as expected from a system where GH signaling is maximal. Indeed, GH concentrations in active acromegaly are well beyond the dose–response curve that can be observed, for example, in short children treated with supraphysiological doses of GH (11). Anyway, this hypothesis requires to be tested in a prospective manner during acromegaly follow-up, since different therapeutic modalities and their variable duration (very difficult to address in a retrospective evaluation) may also impact the post-treatment GH/IGF1 relationship.

In keeping with the known effect of gender on the GH/IGF1 axis in untreated acromegaly (2), we observed a tendency of association between sex and IGF1 concentrations. Oral estrogen treatment, which is known to influence the GH/IGF1 pathway (26), was only used in four patients and did not interfere with the results. In acromegaly, the GH and IGF1 excess leads to a preferential reduction in visceral and s.c. fat, whereas intramuscular fat (27) and fat-free mass (28) increase. The relationship between GH/IGF1 status and body composition is therefore complex. In our study, BMI was not related to IGF1 levels. Finally, at variance with the study of Mercado *et al.* the prevalence of the diabetes mellitus was not associated with *GHR* genotypes.

In conclusion, the *GHR* genotype does not affect the GH/IGF1 relationship in patients with untreated acromegaly. Given the critical role of circulating GH and IGF1 levels in acromegaly patient management (1, 29), further investigations are needed to determine the contribution of other genetic factors to GH/IGF1 pathway modulation.

Declaration of interest

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