D3 growth hormone receptor polymorphism is not associated with IGF-1 levels in untreated acromegaly.

Peter Kamenicky, Christine Dos Santos, Consuelo Espinosa, Sylvie Salenave, Françoise Galland, Yves Le Bouc, Patrick Maison, Pierre Bougnères and Philippe Chanson


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Corresponding author:
Philippe Chanson MD,
Service d’Endocrinologie et Maladies de la Reproduction, Hôpital Bicêtre,
78 rue du Général Leclerc,
F-94 275 Le Kremlin-Bicêtre, France,
e-mail: philippe.chanson@bct.aphp.fr

Short title: Growth Hormone Receptor Polymorphism & Acromegaly
Abstract

Context: A discrepancy between serum GH and IGF-1 concentrations is frequent in patients with acromegaly. Here we examined whether the exon 3-deleted (d3) growth hormone receptor (GHR) variant, which has been linked to increased responsiveness to GH treatment in short children, influences the GH/IGF-1 relationship in patients with acromegaly.

Objective: To study the possible influence of the GHR genotype on the GH/IGF-1 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 IGF-1 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 IGF-1 concentrations did not differ among the three groups. The linear correlation between GH and IGF-1 concentrations was similar in the three genotypic groups.

Conclusions: The exon-3 GHR genotype does not affect the GH/IGF-1 relationship in untreated acromegalic patients with high circulating GH and IGF-1 levels.
**Introduction**

Randomly measured serum GH and IGF-1 concentrations are currently used as indexes of acromegaly disease activity \(^1\). The relationship between log\(_{10}\) serum GH and serum IGF-1 concentrations is linear in patients with active disease \(^2\,^3\), but discordant serum GH and IGF-1 levels are observed in many acromegalic patients \(^2\,^4\,^6\). Although the serum IGF-1 level is mainly dependent on the GH level in acromegaly, IGF-1 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 (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 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/IGF-1 relationship in a cohort of 105 untreated acromegalic patients.

**Patients and Methods**

**Study population**

We reviewed data on 105 acromegalic patients (57 women, 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 IGF-1 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, 22 (21%) patients with a pituitary microadenoma. Forty-four patients (42%) had anterior pituitary hormone deficits, 9 of them had panhypopituitarism. Four patients were receiving oral estrogen, 2 transdermal estrogen, and 1 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 IGF-1 concentrations were measured as described in [24]. Briefly, until 1998, sera were gel-filtered on Ultrogel AcA54 columns in acetic acid. RIA was used for IGF-1, with a specific polyclonal antihuman IGF-1 antibody. Recombinant human IGF-1 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 CVs were 4.8 and 10%, respectively. After 1998 the same IGF-1 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 N 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 IGF-1 concentrations with this method were the same as with the previous method. Thus, throughout this retrospective study, reference IGF-1 concentrations in our laboratory remained unchanged: normal–for-age serum levels of IGF-1 (mean ± SD in µg/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 IGF-1 values were calculated with age-specific reference ranges for our IGF-1 assay (IGF-1% = patient’s IGF-1/age-specific upper limit x 100; the age-specific upper limit = mean IGF-1 normal for age and sex + 2SD). We did not use individual IGF-1 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 IGF-1 values exceeded 5, 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, d3/d3) was determined by simple multiplex PCR as described in [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 ± SEM. For correlation analyses, serum GH concentrations were expressed as log_{10} values. Data were analyzed with Prism version 4 and SAS software. The level of significance was set at $P = 0.05$. Groups were compared with chi-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 IGF-1 concentration, we fitted a multiple linear regression model with age, gender, 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 difference in gender, height, BMI, percentage of macroadenomas, frequency of impaired glucose tolerance / diabetes mellitus, frequency of arterial hypertension or the pretreatment GH and IGF-1 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 IGF-1 concentrations was observed in the three groups (Figure 1) according to closely similar equations. Similar linear associations were found when IGF-1 values were adjusted for age. The multiple regression model showed that IGF-1 concentrations 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 IGF-1 concentrations are used as indexes of disease activity in acromegaly, factors potentially influencing the GH/IGF-1 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/IGF-1 relationship in patients with untreated acromegaly. Two recent studies have previously examined the impact of GHR variants on serum GH and IGF-1 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 IGF-1 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 IGF-1 and to develop acromegalic symptoms. In contrast, Mercado et al. found no difference in GH or IGF-1 concentrations in 152 acromegalic patients at diagnosis, but d3-GHR carrying patients presented with higher IGF-1 concentrations after acromegaly treatment. Interestingly, the exon 3 deletion was a stronger predictor of persistently elevated IGF-1 concentrations during post-treatment follow-up than age at diagnosis, gender and even baseline GH and IGF-1 levels or tumor size.
Surprisingly, the expected correlation between IGF-1 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 found in previous studies.$^{10, 23}$ At variance with the findings of Schmid et al.$^{10}$ and in accordance with the recent study of Mercado et al.$^{23}$ we observed no difference in baseline GH and IGF-1 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 IGF-1 and log$_{10}$ 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/IGF-1 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/IGF-1 relationship.

In keeping with the known effect of gender on the GH/IGF-1 axis in untreated acromegaly$^{2}$, we observed a tendency of association between sex and IGF-1 concentrations. Oral estrogen treatment, which is known to influence the GH/IGF-1 pathway$^{26}$ was only used in 4 patients and did not interfere with the results. In acromegaly the GH and IGF-1 excess leads to a preferential reduction in visceral and subcutaneous fat, whereas intramuscular fat$^{27}$ and fat free mass$^{28}$ increase. The relation between GH/IGF-1 status and body composition is therefore complex. In our study, BMI was not related to IGF-1 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/IGF-1 relationship in patients with untreated acromegaly. Given the critical role of circulating GH and IGF-1 levels in acromegaly patient management$^{1, 29}$, further investigations are needed to determine the contribution of other genetic factors to GH/IGF-1 pathway modulation.
Acknowledgments

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Declaration of Interest

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References

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Legends of Figures

Figure 1: Association between baseline IGF-1 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: IGF-1 = 377log$_{10}$ GH + 411, R = 0.50, P < 0.0001; d3/fl: IGF-1 = 365log$_{10}$ GH + 391, R = 0.53, P < 0.001; d3/d3: IGF-1 = 346log$_{10}$ GH + 448, R = 0.46, P < 0.05)
Figure 1
<table>
<thead>
<tr>
<th>Genotype</th>
<th>fl/fl</th>
<th>fl/d3</th>
<th>d3/d3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (105)</td>
<td>54</td>
<td>31</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>46 ± 2</td>
<td>44.5 ± 2.4</td>
<td>35.7 ± 2.3</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>33/21</td>
<td>13/18</td>
<td>11/9</td>
<td>ns</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.0 ± 1.4</td>
<td>172.7 ± 1.5</td>
<td>173.7 ± 2.5</td>
<td>ns</td>
</tr>
<tr>
<td>BMI</td>
<td>26.5 ± 0.6</td>
<td>27.5 ± 0.6</td>
<td>26.2 ± 0.8</td>
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<tr>
<td>Macroadenoma (%)</td>
<td>81</td>
<td>77</td>
<td>75</td>
<td>ns</td>
</tr>
<tr>
<td>Pituitary deficiency (%)</td>
<td>43</td>
<td>52</td>
<td>25</td>
<td>ns</td>
</tr>
<tr>
<td>Diabetes / IGT (%)</td>
<td>37</td>
<td>39</td>
<td>35</td>
<td>ns</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>33</td>
<td>39</td>
<td>25</td>
<td>ns</td>
</tr>
<tr>
<td>GH (mIU/L)</td>
<td>63.7 ± 10.1</td>
<td>74.7 ± 20.3</td>
<td>93.8 ± 26.4</td>
<td>ns</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>992.4 ± 52.4</td>
<td>957.0 ±75.6</td>
<td>1029.0 ±95.7</td>
<td>ns</td>
</tr>
<tr>
<td>IGF-1 (%)</td>
<td>289.2 ± 16.1</td>
<td>289.2 ±28.4</td>
<td>284.3 ± 25.4</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are means ± SEM; IGF-1 (%) is the percentage of the upper limit (mean IGF-1 + 2SD) of the age-specific references range of IGF-1. IGT: impaired glucose tolerance.