

Growth Hormone (GH) Receptor Isoform in Acromegaly: Lower Concentrations of GH but Not Insulin-Like Growth Factor-I in Patients with a Genomic Deletion of Exon 3 in the GH Receptor Gene

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Background: A genomic deletion of exon 3 (d3-GHR) of the growth hormone (GH) receptor (GHR) has been linked to the effectiveness of GH therapy in children with GH deficiency. Carriers of the d3-GHR genotype had higher GH-induced growth rates than children homozygous for the full-length (fl)-GHR. The aim of this study was to test whether the relationship between GH and insulin-like growth factor-1 (IGF-1) concentrations is influenced by the GHR genotype in patients with acromegaly.

Methods: Study participants were 44 adult patients with established diagnosis of acromegaly. The genotype of the GHR was determined in leukocyte DNA from peripheral blood. Clinical and biochemical findings at the time of diagnosis of acromegaly were obtained from the medical records of the patients.

Results: fl-GHR homozygosity was found in 22 (50%) of patients, and 22 (50%) of patients had at least 1 d3 allele (d3-GHR). Demographic and clinical characteristics (age, height, weight, estimated duration of disease, and

mean tumor size) of the 2 groups were comparable. Median (range) serum IGF-1 concentrations at the time of diagnosis were 670 (447–1443) $\mu\text{g/L}$ in the fl-GHR group and 840 (342–1494) $\mu\text{g/L}$ in the d3-GHR group ($P = \text{not significant}$). Basal GH concentrations were higher in the fl-GHR group [29.7 (3.8–159) $\mu\text{g/L}$] than in the d3-GHR group [8.4 (2.6–74) $\mu\text{g/L}$, $P = 0.002$], and so were mean (30.4 vs 6.1 $\mu\text{g/L}$, $P = 0.005$) and nadir (20.5 vs 5.1 $\mu\text{g/L}$, $P = 0.003$) GH concentrations during an oral glucose tolerance test.

Conclusions: The GHR fl/d3 genotype modulates the relationship between GH and IGF-1 concentrations in patients presenting with acromegaly.

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Acromegaly is a rare disorder caused by an excess of growth hormone (GH)⁵ (1), usually due to a pituitary tumor. Specific clinical signs, such as acral growth, characterize the condition. The measurement of GH during an oral glucose tolerance test (oGTT) is the gold standard for determining GH excess; in patients with acromegaly, GH concentrations are not suppressed to $<1 \mu\text{g/L}$ by oral glucose (2). Insulin-like growth factor-1 (IGF-1), a marker and mediator of GH action, is increased by GH; therefore, screening for acromegaly is often performed by measurement of IGF-1 and random GH concentrations. In acromegaly, log GH concentrations are directly correlated with IGF-1 concentrations (3, 4).

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⁵ Nonstandard abbreviations: GH, growth hormone; oGTT, oral glucose tolerance test; IGF-1, insulin-like growth factor-1; GHR, GH receptor; d3-GHR, polymorphism in the human GHR gene resulting from a genomic deletion of exon 3; fl, full-length.

The response to both exogenous and endogenous GH differs between individuals and is influenced by many variables, including sex (5–7). Women with GH deficiency require higher doses of replacement GH than men (5, 6). Among patients with acromegaly, women were found to have higher GH concentrations than men at any given IGF-1 concentration (7). Another potential factor is a polymorphism in the human GH receptor (GHR) gene resulting from a genomic deletion of exon 3 (d3-GHR) (8). The frequency of the d3-GHR genotype is quite high, with approximately half of the individuals in most populations tested being carriers of the d3-GHR. Although initial studies comparing d3-GHR and full-length (fl)-GHR indicated no differences in GH binding by the 2 isoforms, subsequent analysis using reporter constructs to assess transcriptional activity clearly showed that the deletion of exon 3 affected the functional properties of the GHR (9). In the same study, young carriers of an allele encoding the d3-GHR have been found to be more responsive (in terms of growth) to GH administration than individuals homozygous for the fl-GHR, wild-type GHR (9). This finding was confirmed in children with Turner syndrome and short-stature small-for-gestational-age (10, 11) but not in short-stature non-GH-deficient small-for-gestational-age children, or in children with isolated GH deficiency (12, 13). In healthy individuals, differences in GH sensitivity may be compensated for by adjusted GH secretion. Because feedback control of GH secretion by IGF-1 is grossly attenuated in acromegaly (14), we tested whether the relationship between GH and IGF-1 was GHR isoform-specific (d3-GHR vs fl-GHR) in these patients.

Materials and Methods

PATIENTS

A total of 60 patients with established diagnosis of acromegaly who had been treated at the Division of Endocrinology and Diabetes at the University Hospital in Zurich from January 1994 to December 2004 (selected from 64 known during that period, provided address and charts were available) were invited to participate in the study. Four patients declined to participate, and 12 patients who consented were excluded because of missing baseline data. Thus a total of 44 patients were included, from whom we obtained written informed consent and determined baseline hormone values at our own laboratory. The diagnosis of acromegaly in these patients was determined by clinical findings of increased IGF-1 and GH, the latter nonsuppressible to $<1 \mu\text{g/L}$ during an oGTT (data available from 36 patients). Genomic DNA was obtained from peripheral blood cells. Demographic and clinical data at diagnosis of acromegaly were obtained from the medical records. The Ethics Committee of the University Hospital of Zurich approved the study. Written informed consent was obtained from all patients.

LABORATORY METHODS

For the determination of IGF-1, carrier proteins were removed by Sep-Pak® chromatography according to the instructions of the supplier (Waters Associates), and IGF-1 was determined by RIA. GH was determined by an immunoradiometric assay (hGH-RIACT, CIS Bio international, Oris Industries) with 2 monoclonal antibodies prepared against sterically remote antigenic sites on the hGH molecule, with a lower detection limit reported to be $0.02 \mu\text{g/L}$ (15, 16). As determined in our laboratory, during the time of the study intraassay CVs were 2.2% at a GH concentration of $2.1 \mu\text{g/L}$ and 2.1% at a GH concentration of $23.4 \mu\text{g/L}$, and interassay CVs were 7.7% at a GH concentration of $2.1 \mu\text{g/L}$ and 6.8% at a GH concentration of $23.4 \mu\text{g/L}$.

Genomic DNA was isolated from peripheral blood with the Qiamp DNA Blood Mini Kit (Qiagen GmbH). Genotyping for the fl/d3 polymorphism of the *GHR*⁶ gene was performed on LightCycler PCR (Roche Diagnostics AG) with melting analysis and using the GHR fl/d3 ToolSet (Genes-4U AG) according to the instructions of the manufacturer. This ToolSet is specifically designed for genotyping the human *GHR* gene for presence of the fl allele (fl-GHR) or d3-GHR. Primer pair and fluorescent detection/anchor probes have been optimized for specific amplification of a 169-bp segment of either repeat 1 (5' of exon 3) in the case of fl-GHR or repeat 2 (3' of exon 3) in the case of d3-GHR. Repeats 1 and 2 are identical except for 3 single-nucleotide polymorphisms. The single nucleotide polymorphism C14G of the repeat is typed by melting curve analysis and thus allows differentiation of the fl-GHR (C) from the d3-GHR (G) allele (8).

STATISTICAL ANALYSIS

Data are presented as mean (SD) or median (range). GH concentrations were logarithmically transformed because of highly skewed distributions. Evaluations were performed using Statistica version 6. A *P*-value <0.05 was considered statistically significant. Comparisons of data were done using the χ^2 test (numbers), unpaired Student *t*-test (means), or Mann-Whitney *U*-test (medians). Multiple regression analyses were performed on log serum GH basal and serum IGF-1 to exclude possible confounding effects; variables with uncertain influence (*P* >0.10) were excluded during analysis using a stepwise backward elimination procedure.

Results

PATIENTS

Study patients included 25 females and 19 males. Mean (SD) age at diagnosis of acromegaly was 44 (13) years and body mass index was $26.8 (4.5) \text{ kg/m}^2$. Acromegalic symptoms had been present for 6.5 (5) years. Imaging techniques (MRI or CT scans) revealed pituitary

⁶ Human gene: *GHR*, growth hormone receptor.

macroadenomas (≥ 10 mm in diameter) in 37 (84%) and microadenomas in 7 (16%) of patients.

We found 22 (50%) of patients to be homozygous carriers of fl-GHR and 22 (50%) of patients to carry at least 1 allele of the d3-GHR isoform. Patients were assigned to 2 groups, carriers homozygous for the fl-GHR (fl-GHR group) and carriers of the d3-GHR (combined 18 hetero- and 4 homozygous, d3-GHR group). Demographic and clinical characteristics at diagnosis did not differ significantly in the 2 groups (see Table 1 in the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol53/issue8>).

GH AND IGF-1 CONCENTRATIONS

At the time of diagnosis of acromegaly, median (total range) IGF-1 concentrations in the fl-GHR group [670 (447–1443) $\mu\text{g/L}$] and in the d3-GHR group [840 (342–1494) $\mu\text{g/L}$] were comparable ($P = 0.85$). In contrast, basal GH was significantly higher in the fl-GHR group [29.7 (3.8–159) $\mu\text{g/L}$] than in the d3-GHR group [8.4 (2.6–74) $\mu\text{g/L}$; $P = 0.002$]. Among the 36 patients for whom GH values during the 75-g oGTT were available, nadir and mean GH concentrations during the test were significantly higher in carriers of the fl-GHR than in carriers of the d3-GHR. Nadir GH concentrations were 20.5 (1.8–75) in the fl-GHR group and 5.1 (1.6–50) in the d3-GHR group ($P = 0.003$). Mean GH concentrations during the oGTT were 30.4 (3.4–100) in carriers of the fl-GHR and 6.1 (3.5–74) in carriers of the 3-GHR ($P = 0.005$).

Basal GH concentrations were slightly higher in females [17.3 (4.3–159) $\mu\text{g/L}$] than in males [16.0 (2.6–75) $\mu\text{g/L}$, $P = 0.16$], whereas IGF-1 tended to be lower in females [630 (375–1494) $\mu\text{g/L}$] than in males [867 (342–1338) $\mu\text{g/L}$, $P = 0.31$].

The relationship between log serum GH concentrations and IGF-1 concentrations is shown in Fig. 1. Higher log GH basal concentrations were correlated with higher IGF-1 concentrations. At any given serum IGF-1 concentration, log serum GH concentrations were lower in carriers of the d3-GHR (18 patients were heterozygous and 4 homozygous) compared to patients carrying only the fl-GHR isoform. For a given log GH concentration, carriers of the d3-GHR had higher IGF-1 concentrations compared with carriers of the fl-GHR, i.e., their curve was shifted to the left.

A stepwise multiple regression analysis on log serum GH concentrations and IGF-1 concentrations is shown in Table 1. The regression analysis on log serum GH concentrations showed a significant negative correlation with the d3-GHR isoform (β -coefficient -0.5 , $P = 0.0001$). Log serum GH concentrations were also significantly correlated with IGF-1 concentrations. The negative correlation of male sex and body mass index with log GH concentrations did not reach statistical significance, and no association was found with age at diagnosis, estimated duration of disease at diagnosis, diabetes, arterial hypertension, or tumor size.

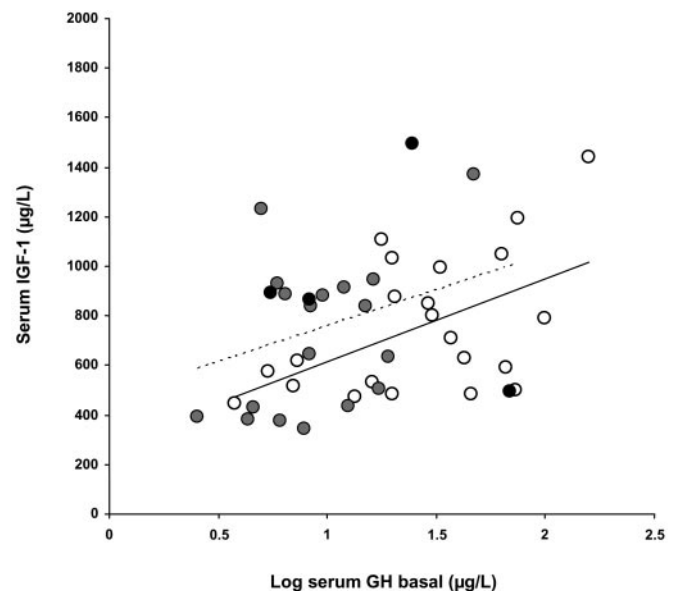


Fig. 1. The relationship between serum GH and IGF-1 in 44 patients at diagnosis of acromegaly.

Serum IGF-1 is plotted as a function of \log_{10} GH. The 22 carriers of the d3-GHR are shown as filled (4 homozygous patients, black and 18 heterozygous patients, gray) circles (broken line), the 22 carriers of the fl-GHR as open circles (solid line).

The multiple regression analysis on IGF-1 concentrations and GHR genotype did not reach statistical significance, but showed a significant positive correlation with log serum GH concentrations, male sex, estimated duration of disease until the time of diagnosis, and diabetes. A striking negative correlation was found with age.

Discussion

Our findings describe an impact of the GHR genotype on the relationship between GH and IGF-1. For the first time, a polymorphism in the GHR was observed to influence endogenous GH concentrations in adult patients with acromegaly. It is important to point out that our analysis focused on baseline data, i.e., on the relationship between GH and IGF-1 at the time when the diagnosis of acromegaly was suspected and finally confirmed; the patients were not treated for their disease. Apparently, lower GH concentrations are required for patients carrying a d3-GHR allele to produce a given increase in serum IGF-1 concentrations and to develop acromegalic symptoms. It has been reported that disease activity may be more closely related to IGF-1 than to GH (17). Most clinical signs and symptoms that lead to suspected acromegaly may be mediated by IGF-1 (rather than caused by GH itself), and similar IGF-1 concentrations in carriers of the d3-GHR and fl-GHR were found at the time of diagnosis. GH concentrations (basal as well as nadir and mean GH in an oGTT) were lower in carriers of the d3-GHR, suggesting that less GH was required for disease development, which appeared to progress (estimated duration of disease) at comparable rates, irrespective of GHR

Table 1. Stepwise multiple regression analysis on log serum GH basal and serum IGF-1 concentration with clinical and biochemical variables and GHR genotype.

Predictor variables	Log serum GH basal		Serum IGF-1	
	β -coefficient	P value	β -coefficient	P value
GHR exon 3 deletion	−0.50	0.0001	0.24	0.08
Log serum GH basal, $\mu\text{g/L}$			0.31	0.025
Serum IGF-1, $\mu\text{g/L}$	0.36	0.004		
Male sex	−0.24	0.06	0.27	0.041
Body mass index, kg/m^2	−0.21	0.08	−0.10	0.43
Age at diagnosis, year	0.08	0.66	−0.62	0.00005
Disease duration	0.02	0.90	0.50	0.0003
Diabetes	−0.06	0.62	0.25	0.029
Arterial hypertension	−0.11	0.41	0.15	0.25
Tumor size	−0.10	0.44	0.07	0.54

isoforms. Admittedly, it is rather difficult to estimate (in retrospect) the duration of symptomatic disease based on history rather than biochemistry. Tumor size was similar in the carriers of the d3-GHR and fl-GHR isoforms (see Table 1 in the online Data Supplement). Moreover, tumor size was not related to GH concentrations (Table 1).

Stepwise multiple regression analysis of log serum GH basal concentrations confirmed a significant influence of the GHR genotype on the relationship between GH and IGF-1 concentrations, even when additional variables with known effects on GH concentrations were considered (such as sex or age of patients). Although the correlation between d3-GHR and serum IGF-1 did not reach statistical significance ($P = 0.08$), carriers of the d3-GHR tended to have higher serum IGF-1 concentrations compared with carriers of the fl-GHR isoform. The known influence of sex on serum GH concentrations in patients with acromegaly (7) could not be confirmed in our study, which showed only a trend for higher GH (and lower IGF-1) concentrations in women than in men. In addition, the stepwise multiple regression analysis confirmed the negative correlation of age with serum IGF-1 concentrations, i.e., lower IGF-1 concentrations in older patients with acromegaly. Furthermore, patients with a longer estimated duration of the disease had higher IGF-1 concentrations.

The lower GH concentrations in d3-GHR carriers and the shift of the dose–response curve (Fig. 1) to the left suggest improved signaling of GH through the d3-GHR isoform (9). Another possible, though less likely, theory for our findings is that there is an association with other unknown genetic variations that accounts for enhanced IGF-1 feedback.

Our results may have implications with regard to the interpretation of GH concentrations and distinguishing between health and disease in patients with acromegaly. The gold standard test to evaluate diagnosis and cure of acromegaly remains nonsuppressible or suppressible GH during an oGTT. The cutoff nadir of suppression of serum GH concentrations is $<1 \mu\text{g/L}$ after a 75-g glucose load; alternatively, it has been proposed that a suppression test

is not required if a GH random value of $<0.4 \mu\text{g/L}$ is found (1, 2, 18). Our findings suggest that the utility of this criterion is limited not only by problems related to GH assays (1, 18, 19) but also by the fact that actions of excessive GH, rather than a given increase of the GH concentration per se, cause morbidity. Whether patients treated for acromegaly can be considered cured should not be based simply on the provisional fixed cutoff, as proposed in a consensus (2); it is likely that fair decision limits of GH nadir values in an oGTT are specific not only for the assay and patient sex (19) but also for the GHR isoform. This theory should be investigated further in future studies.

In conclusion, our findings demonstrate that the GHR genotype (specifically, the deletion of exon 3) modulates the relationship between GH and IGF-1 concentrations in patients presenting with acromegaly. GH concentrations are lower for any given serum IGF-1 concentration in carriers of the d3-GHR isoform compared to carriers of the fl-GHR isoform. Thus, in patients carrying a GHR d3 allele, on average, only half the amount of GH is required to cause the same increase of IGF-1 and comparable clinical expression of the disease.

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