Influence of Growth Hormone (GH) Receptor Deletion of Exon 3 and Full-Length Isoforms on GH Response and Final Height in Patients with Severe GH Deficiency

Barbara Räz,* Marco Janner,* Vibor Petkovic, Didier Lochmatter, Andrée Eblé, Mehul T. Dattani, Peter C. Hindmarsh, Christa E. Flück, and Primus E. Mullis

University Children's Hospital (B.R., M.J., V.P., D.L., A.E., C.E.F., P.E.M.), Pediatric Endocrinology, Diabetology & Metabolism, University Bern, CH-3010 Bern, Switzerland; and Developmental Endocrinology Research Group (M.T.D., P.C.H., P.E.M.), Clinical and Molecular Genetics Unit, Institute of Child Health, London WC1N 1EH, United Kingdom

Context: A polymorphism of the GH receptor (GHR) gene resulting in genomic deletion of exon 3 (GHR-d3) has been associated with responsiveness to GH therapy. However, the data reported so far do vary according to the underlying condition, replacement dose, and duration of the treatment.

Objective, Design: The aim of this study was to analyze the impact of the GHR genotypes in terms of the initial height velocity (HV) resulting from treatment and the impact upon adult height in patients suffering from severe isolated GH deficiency.

Controls, Patients, Setting: A total of 181 subjects (peak stimulated GH $\leq$ 2 ng/ml) were studied. In addition, GHR genotype frequency was compared with a healthy adult control group.

Interventions: Based on the various GHR genotypes, HV, effect of recombinant human GH dose used, and final height were analyzed.

Main Outcome Measures, Results: In the 181 subjects after the first two yr on recombinant human GH treatment, HV SD score (SDS) as well as height gain were significantly greater in subjects with the GHR-d3/d3 genotype when compared with the subjects presenting with the GHR-full-length/full-length genotype ($P < 0.05$). A GHR-d3 allele dose-dependent effect was found for both HV SDS ($r = 0.72$) and height gain ($r = 0.77$). However, there was no significant difference in final adult height and height SDS according to the exon-3 genotypes.

Conclusions: Our results indicate that in patients with severe isolated GH deficiency, although the GHR genotype might play a role in GH responsiveness, at least at the beginning of treatment, there is no effect on final height. **(*J Clin Endocrinol Metab* 93: 974–980, 2008)**

GH deficiency (GHD) is heterogeneous in terms of etiology, pathogenesis, and age at diagnosis. Improvement in adult height is the major aim of treating GH-deficient children with recombinant human GH (rhGH) (1). The final height attained as a result of intervention is influenced, in part, by the dose, injection frequency, and duration of rhGH therapy. Despite optimization of these factors, a proportion of GH-deficient patients do not reach their target height (1, 2). A number of mathematical models for predicting growth and final outcome has been proposed, enabling the clinician to "personalize" the growth promoting therapy on the grounds of efficacy and economy (2–8). However, a range of responsiveness to rhGH exists, even when

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2007-1382 Received June 21, 2007. Accepted November 13, 2007.

First Published Online November 20, 2007

* B.R. and M.J. contributed equally to the work.

Abbreviations: BA, Bone age; d3, deletion of exon 3; fl, full-length; GHD, GH deficiency; GHR, GH receptor; HV, height velocity; IGHD, isolated GH deficiency; ISS, idiopathic short stature; rhGH, recombinant human GH; SDS, SD score; SGA, short for gestational age; TW-2, Tanner-Whitehouse-2 prediction.

factors such as statural deficit before treatment, bone "age," midparental height, injection frequency, and dose of rhGH are considered.

It is likely that underlying genetic and epigenetic factors may explain this individual variability of GH response. Based on animal knockout and human mutational analysis, the most likely candidates are factors/genes affecting IGF generation (9). A polymorphism in the GH receptor (GHR) gene leading to retention [full-length (fl)] or deletion of exon 3 (d3), which encodes a 22-amino acid residue sequence in the extracellular domain (10, 11), has recently been associated with the degree of height increase in response to GH replacement in children born short for gestational age (SGA), those with idiopathic short stature (ISS) (12), and in a GH-deficient population (13). Patients with at least one d3 allele had a significantly better first-year response, leading to an improved adult height on GH treatment than patients with homozygosity for GHR-fl (13). However, reported studies are not all consistent, which may reflect differing populations and conditions (14–19). This makes the application of a pharmacogenetics/genomic approach to therapy problematic.

The aim of this study was to evaluate the impact of the exon-3 GHR genotype on short-term growth response and final height in severely GH-deficient children treated with rhGH. We now report the results of the short-term growth response of 181 severely GH-deficient children treated for 4 yr (study A) according to the exon-3 GHR genotype and, in addition, the results of the long-term growth response of 95 severely GH-deficient children treated to final height (study B).

Subjects and Methods

Subjects

Study A: follow-up of 4 yr [median rhGH; 31.5 μg/kg·d (range 24–38); 20 IU/m²·wk (range 15–24)]

A total of 181 subjects with severe growth retardation due to isolated GHD (IGHD) was studied. Auxological data are shown in Table 1. These subjects belonged to 124 nonconsanguineous, unrelated families of Caucasian origin. Diagnostic criteria included a pretreatment height of less than −3.0 SD score (SDS), decreased height velocity (HV) (< −2.5 SDS), retarded bone age (BA), normal karyotype, normal prolactin concentration and thyroid function, and peak GH concentrations less than or equal to 2 ng/ml after two stimulation tests (20–23). The remainder of the hypothalamic-pituitary axis tested normal (23). Standard auxological assessment was performed (21, 22). None of the affected patients had evidence of an organic disease, psychosocial deprivation, or any eating disorder, and all had normal renal and hepatic function. The BA was estimated according to the methods of Greulich and Pyle (24) and Tanner *et al.* (25). No other hormonal deficiency occurred over the study period. Informed consent was obtained from parents and all family members studied. The study was approved by the Clinical Research Ethics Committee.

Study B: up to final height [median rhGH; 27.0 μg/kg·d (range 24–31); 17.0 IU/m²·wk (range 15–19.5)]

From study A, we identified a subgroup of 95 subjects with severe growth retardation secondary to IGHD, and who had reached final height. Auxological data are shown in Table 2. These subjects belonged to 63 nonconsanguineous, unrelated families of Caucasian origin. Diagnostic criteria and stimulation tests are described previously. No other hormonal deficiency occurred over the study period. Informed consent was obtained from parents and all family members studied. The study was approved by the Clinical Research Ethics Committee.

Adult control population

A control population composed of 144 healthy adult males recruited at the medical checkup before military service in Switzerland (age range 19–22 yr), and of 67 healthy women recruited at the University Hospital in Bern, Switzerland (age range 20–50 yr), was studied. All control subjects were of normal height (≤ 2 and ≥ −2 SDS) (20). None of the subjects had a family history of pathological short stature, or had received any therapy with rhGH or any other anabolic agent. The study was approved by the Clinical Research Ethics Committee.

Hormonal assays

Pituitary stimulation tests were performed as described previously (23, 26). Over the years, GH, IGF-I, TSH, LH, FSH, and prolactin were measured using various assays, as previously described. The correlations among the different tests were between r = 0.83 and 0.97.

GH assays

GH was measured using an immunoradiometric assay, HGH MAIAclone (Biodata Diagnostics, Freiburg, Germany), which incorporates two high-affinity monoclonal antibodies. The interassay coefficients of variation were 2.3, 2.4, and 2.2% at 2, 9, and 24 ng/ml, respectively. The intraassay coefficients of variation were 2, 1.7, and 1.7% at 2, 9, and 24 ng/ml.

TABLE 1. Auxological data and characteristics of subjects studied at commencement of rhGH replacement therapy [median (range) rhGH dose: 31.5 (24–38) μg/kg·d]

	Males (n = 98)			Females (n = 83)		
Characteristics	fl/fl 50 (51.0%)	fl/d3 37 (37.8%)	d3/d3 11 (11.2%)	fl/fl 40 (48.2%)	fl/d3 33 (39.7%)	d3/d3 10 (12.1%)
CA (yr)	6.7 ± 2.2	6.2 ± 1.8	6.5 ± 1.6	6.7 ± 2.1	6.8 ± 2.0	5.9 ± 1.6
CA − BA (yr)	3.3 ± 1.3	3.2 ± 1.3	3.3 ± 1.8	3.4 ± 1.4	3.0 ± 1.2	3.0 ± 2.0
Ht SDS	−4.1 ± 0.6	−4.0 ± 0.5	−3.8 ± 0.5	−4.2 ± 0.7	−3.9 ± 0.3	−3.9 ± 0.4
Midparental target Ht (cm)	175.2 ± 4.7	174.8 ± 4.0	175.1 ± 4.1	163.6 ± 4.7	163.0 ± 3.9	162.7 ± 3.8
Midparental target Ht (SDS)	−0.2 ± 0.8	−0.3 ± 0.7	−0.2 ± 0.7	−0.3 ± 0.8	−0.4 ± 0.7	−0.4 ± 0.6
Peak GH (ng/ml)	0.8 ± 0.4	0.7 ± 0.3	0.9 ± 0.4	0.8 ± 0.4	1.1 ± 0.5	0.7 ± 0.3
IGF-I SDS	−3.3 ± 1.2	−3.4 ± 1.7	−3.8 ± 1.4	−3.4 ± 1.5	−3.6 ± 1.6	−3.7 ± 1.4
TW-2 predicted adult Ht (cm)	156.9 ± 7.5	156.3 ± 8.0	157.4 ± 7.6	141.8 ± 7.3	144.1 ± 6.6	142.5 ± 7.0
TW-2 predicted adult Ht (SDS)	−3.8 ± 1.5	−3.9 ± 1.8	−3.7 ± 1.5	−3.6 ± 1.1	−3.2 ± 0.9	−3.4 ± 1.3

CA, Chronological age; Ht, height.

TABLE 2. Auxological data and characteristics of subjects studied at commencement of rhGH replacement therapy (final height study, mean ± SD rhGH dose: 26.5 ± 1.3 μg/kg·d)

Characteristics	Males (n = 52)			Females (n = 43)		
	fl/fl 27 (51.9%)	fl/d3 19 (36.5%)	d3/d3 6 (11.6%)	fl/fl 21 (48.8%)	fl/d3 17 (39.5%)	d3/d3 5 (11.7%)
Beginning rhGH therapy						
CA (yr)	7.3 ± 2.1	7.0 ± 1.7	7.2 ± 1.4	7.2 ± 2.0	7.3 ± 1.9	6.0 ± 1.3
CA − BA (yr)	3.4 ± 1.3	3.2 ± 1.2	3.3 ± 1.8	3.6 ± 1.2	3.3 ± 1.1	3.0 ± 2.0
Ht SDS	−4.2 ± 0.5	−3.9 ± 0.5	−3.8 ± 0.3	−4.3 ± 0.6	−3.9 ± 0.3	−3.9 ± 0.4
Midparental target Ht (cm)	176.6 ± 5.0	175.3 ± 4.2	177.2 ± 4.0	164.2 ± 4.6	162.9 ± 4.1	163.2 ± 3.5
Midparental target Ht (SDS)	0.2 ± 0.8	−0.3 ± 0.6	0.2 ± 0.7	−0.2 ± 0.8	−0.4 ± 0.6	−0.3 ± 0.6
Peak GH (ng/ml)	0.4 ± 0.3	0.4 ± 0.2	0.5 ± 0.3	0.3 ± 0.2	0.6 ± 0.3	0.5 ± 0.3
IGF-I SDS	−3.4 ± 1.2	−3.3 ± 1.8	−4.1 ± 1.2	−3.5 ± 1.5	−3.8 ± 1.3	−3.8 ± 1.6
TW-2 predicted adult Ht (cm)	157.8 ± 8.0	158.1 ± 7.9	158.2 ± 7.5	142.2 ± 7.4	145.5 ± 6.5	143.4 ± 7.1
TW-2 predicted adult Ht (SDS)	−3.8 ± 1.6	−3.6 ± 1.4	−3.6 ± 1.5	−3.4 ± 1.1	−3.0 ± 0.7	−3.4 ± 1.3
End rhGH therapy						
Duration of rhGH treatment (yr)	11.6 ± 1.8	11.3 ± 2.0	10.7 ± 1.6	11.0 ± 1.8	11.1 ± 2.2	10.9 ± 1.6
Adult Ht (cm)	173.9 ± 6.7	172.9 ± 7.4	174.1 ± 6.5	161.6 ± 6.9	161.1 ± 4.5	160.3 ± 3.8
Adult Ht (SDS)	−0.4 ± 1.0	−0.6 ± 1.2	−0.3 ± 0.9	−0.6 ± 1.3	−0.6 ± 1.2	−0.7 ± 0.7
Adult Ht SDS − midparental target Ht SDS	−0.6 ± 0.7	−0.3 ± 1.0	−0.5 ± 1.1	−0.4 ± 1.2	−0.2 ± 1.1	−0.4 ± 0.9
Age at onset of puberty (yr)[a]	13.0 ± 3.2	12.3 ± 2.8	11.6 ± 2.3	11.5 ± 2.0	10.7 ± 2.2	10.4 ± 1.9
Change in Ht SDS on therapy	3.6 ± 0.9	3.3 ± 0.8	3.4 ± 0.6	3.7 ± 1.2	3.3 ± 1.0	3.2 ± 0.4
Adult Ht − PAH at start of treatment (cm)	16.1 ± 7.2	18.7 ± 6.5	17.3 ± 7.2	19.8 ± 6.7	18.1 ± 7.1	18.8 ± 6.9

CA, Chronological age; Ht, height; PAH, predicted adult height.

[a] Boys, testicle size more than or equal to 4 ml; girls, breast stage Tanner II.

IGF-I assays

IGF-I concentrations were determined by either IGF-I kit (Nichols Institute Diagnostics, Bad Vilbel, Germany) or using the assays described by Blum *et al.* (27).

DNA isolation and GH-1/GHR gene analysis

Genomic DNA was isolated from peripheral leukocytes of subjects and their relatives (28). The entire *GH-1* gene was sequenced as previously described (29, 30). For genotyping of the GHR exon-3 locus, a simple multiplex PCR assay was used as previously described (10). Amplification products were analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide. GHR-fl allele was detected as a single band corresponding to 935 bp and the GHR-d3 allele corresponding to 532 bp, respectively.

Statistical analysis

Qualitative variables are listed in row numbers, frequencies, as well as in percentages. However, quantitative variables are stated in mean values ± SD. Hardy-Weinberg equilibrium was calculated according to standard procedures using χ^2 analysis. Differences for GHR-d3/fl genotype frequencies between isolated GH-deficient subjects, controls, and height SDS groups were analyzed by the χ^2 test. In addition, differences between GHR-d3/fl genotypes for auxological parameters in GH-deficient subjects were assessed using ANOVA Fisher's protected least significant difference test. *P* values less than 0.05 were considered significant. Furthermore, a multiple linear regression analysis assessing the established influential factors influencing its prognostic significance was performed. All statistical analyses were performed using SigmaStat for Windows (version 3.5; Systat Software UK Ltd., London, UK).

Results

Population studies and GHR-genotype frequencies

To compare the affected families with a normal population, the GHR genotypes in 211 unrelated normal Swiss subjects were analyzed (20). Individual allele frequencies of controls and parents of isolated GH-deficient subjects were similar in the three height subgroups, and genotype frequencies reached Hardy-Weinberg equilibrium in all the height subgroups studied. Importantly, the genotype frequency of the isolated GH-deficient subjects, as well as the overall control group, was similar, as it was when the genotype frequencies were compared with the overall parental data (Table 3).

Study A

All patients showed severe IGHD, and sequencing of the entire *GH-1* gene did not reveal any abnormalities. Stimulated GH peaks ranged between 0.3 and 2.0 ng/ml. At the commencement of rhGH replacement therapy, the age of the patients ranged between 4.6 and 9.5 yr. The BA delay ranged between 0.8 and 5.1 yr. Chronological age, BA delay, height SDS, midparental target height SDS, and Tanner-Whitehouse-2 prediction (TW-2) adult height SDS were not statistically different between boys and girls or between GHR genotypes (Table 1).

The first-year HV SDS was significantly higher in subjects with the GHR-d3/d3 genotype when compared with the subjects with the GHR-fl/fl genotype ($P < 0.05$) (Table 4). Although, overall, patients carrying the GHR-d3/fl genotype when compared with GHR-fl/fl did not present with a statistically different HV SDS ($P < 0.07$), a significantly higher HV SDS was, however, observed in patients with one or two copies of GHR-d3 when compared with subjects homozygous for GHR-fl/fl ($P < 0.05$); therefore, a GHR-d3 allele dose-dependent effect on HV SDS was found (r = 0.72). In addition, compared with GHR-fl/fl, the height gain after 2-yr treatment expressed in SDS was significantly higher in both girls and boys carrying the GHR-d3/d3 ($P < 0.05$) (Table 4). Furthermore, the GHR-d3 allele dose-dependent

TABLE 3. Genotype frequency among IGHD subjects and their the parents ($2 \times 117 \geq 234$), and among the adult control population

	Height SDS				
	≤2 and >1	≤1 and > −1	≤ −1 and ≥ −2	≤2 and ≥ −2	
	IGHD parents				IGHD subjects
No. of subjects	50	136	62	248	181
Frequency d3/d3 (n)	22.0% (11)	16.9% (23)	14.5% (9)	17.3% (43)	11.6% (21)
Frequency d3/fl (n)	48.1% (24)	47.8% (65)	48.4% (30)	48.0% (119)	38.7% (70)
Frequency fl/fl (n)	29.9% (15)	35.3% (48)	37.1% (23)	34.7% (86)	49.7% (90)
d3 allele frequency	0.43	0.38	0.35	0.39	0.32
fl allele frequency	0.57	0.62	0.65	0.61	0.68
	Adult control population				
No. of subjects (m/f)	38 (30/8)	148 (97/51)	25 (17/8)	211 (144/67)	
Frequency d3/d3 (n)	13.1% (5)	7.4% (11)	8.0% (2)	8.5% (18)	
Frequency d3/fl (n)	47.4% (18)	42.6% (63)	44.0% (11)	43.6% (92)	
Frequency fl/fl (n)	39.5% (15)	50.0% (74)	48.0% (12)	47.9% (101)	
d3 allele frequency	0.37	0.29	0.30	0.30	
fl allele frequency	0.63	0.71	0.70	0.70	

f, Female; m, male.

effect was also present (r = 0.77). Although the growth response during the first 2 yr was better in subjects expressing either the GHR-d3/d3 or the GHR-d3/fl genotype, during the third ($P < 0.05$: males and females) and fourth ($P < 0.05$: females; $P < 0.07$: males) year on treatment, the opposite was noted. Finally, subjects with the GHR-d3/d3 genotype showed a trend toward an earlier start of pubertal development. However, this observation was not statistically significant.

Study B

Of the 181 subjects analyzed in study A, 95 reached final height and have been separately analyzed (Table 2). There was no significant difference in adult height and adult height SDS according to the exon-3 genotypes. All subjects independently of their genotype showed slightly lower adult height SDS compared with midparental height SDS (Table 2).

Therefore, the effect of rhGH replacement therapy at a median dose of 27.0 μg/kg·d did not reveal any difference between the various genotypes in terms of final height.

Multiple linear regression model

We performed multiple linear regression analysis considering the genotype, chronological age, height SDS at start of replacement therapy, BA, duration of treatment, age at puberty, and GH dose. GHR genotype showed a significant relationship ($P < 0.05$) with growth during the first years of replacement therapy, which, however, was not demonstrated at final height (Tables 2 and 4). Furthermore, study B (final height study) did not reveal any variables significantly related to HV.

To study the impact of rhGH dose on growth in subjects with IGHD carrying different GHR isoforms, the study population was divided into two groups: group A (n = 95), 52 males and 43 females, rhGH dose 26.5 ± 1.3 (mean ± SD) μg/kg·d; and group B (n = 86), 46 males and 40 females, rhGH dose 36.5 ± 2.1 μg/kg·d). Importantly, these two groups, although identical in terms of clinical as well as laboratory findings, do not overlap in terms of rhGH dose used, and, therefore, this separate analysis is statistically valid. A relationship between HV SDS in the first-year treatment and GHR genotype (homo- and heterozygosity

TABLE 4. Auxological parameters on rhGH treatment [median (range) rhGH dose: 31.5 (24–38) μg/kg·d]

	Males (n = 98)			Females (n = 83)		
Characteristics	fl/fl 50 (51.0%)	fl/d3 37 (37.8%)	d3/d3 11 (11.2%)	fl/fl 40 (48.2%)	fl/d3 33 (39.7%)	d3/d3 10 (12.1%)
First-year HV-SDS median rhGH: 30.8 μg/kg·d	5.5 ± 1.5	6.0 ± 1.7	6.3 ± 1.8[a]	5.5 ± 1.7	5.7 ± 2.1	6.1 ± 1.6[b]
Second-year HV-SDS median rhGH: 31.3 μg/kg·d	3.9 ± 1.9	4.1 ± 1.6	4.1 ± 1.8	3.6 ± 1.7	3.9 ± 1.8	3.9 ± 1.4
Ht gain (first + second year; SDS)	2.3 ± 0.7	2.4 ± 0.8	2.6 ± 1.0[a]	2.1 ± 0.8	2.4 ± 0.7	2.5 ± 0.4[a]
Third-year HV-SDS median rhGH: 31.9 μg/kg·d	2.0 ± 0.7[c]	1.8 ± 0.8	1.6 ± 0.9	2.0 ± 0.5[c]	1.8 ± 0.6	1.5 ± 0.7
Fourth-year HV-SDS median rhGH: 31.4 μg/kg·d	1.8 ± 0.6	1.6 ± 0.5	1.5 ± 0.7	1.7 ± 0.8[c]	1.3 ± 0.7	1.2 ± 0.6
Ht gain (third to fourth year; SDS)	0.7 ± 0.4	0.6 ± 0.4	0.6 ± 0.3	0.8 ± 0.5	0.6 ± 0.4	0.5 ± 0.3

Ht, Height.

[a] $P < 0.05$ (d3/d3 *vs.* fl/fl).

[b] $P < 0.01$ (d3/d3 *vs.* fl/fl).

[c] $P < 0.05$ (fl/fl *vs.* d3/d3).

for GHR-d3) ($P < 0.01$) was demonstrated in group A. The GHR genotype explained 22% of the variability in HV SDS as well as height gain during the first-year rhGH treatment. Importantly, this relationship could not be found in subjects with the higher rhGH treatment dose (group B). The clinical details are submitted as supplemental files, which are published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

Discussion

In this study we analyzed the impact of GHR genotypes (GHR-d3/d3, GHR-d3/fl, and GHR-fl/fl) on growth response to rhGH replacement therapy in patients suffering from severe IGHD (mean maximal GH peak on GH stimulation tests: 0.9 ng/ml). A total of 181 children were followed (study A; Tables 1 and 4), and prepubertal HVs during the first 4 yr on rhGH replacement (median dose 31.5 μg/kg·d) were assessed. In contrast to previous studies, in our study the subjects with the GHR-d3/d3 and GHR-d3/fl were not only pooled but also separately analyzed (13, 18). Importantly, in the subjects presenting with either GHR-d3/d3 or pooled, so that they had at least one copy of the GHR-d3, an overall significantly better response to the replacement therapy during the first 2-yr therapy was noted, although in the third and fourth year of therapy, this improved HV was observed in those patients with the GHR-fl/fl genotype. In addition, during the first 2 yr on therapy, a GHR-d3 allele-dependent effect on height was found in study A ($r = 0.72$). However, at final height (study B), the effect of rhGH treatment was identical regardless of the specific GHR genotype. It is worth stressing that the difference between final adult height SDS and the midparental target height SDS in our severely GH-deficient patients was similar to that found in a large cohort of Caucasian GH-deficient children recruited from the Kabi International Growth Study (KIGS) database (31). Similar data were previously published by the Genentech Growth Study Group (1) underlining the effectiveness of the rhGH treatment used in our studies. From these data also reporting final height, it can be concluded that in severe GH-deficient subjects, the presence or absence of the GHR-d3 allele has no impact on either baseline phenotype or final height, although a difference in response to rhGH replacement therapy during the first years may be observed depending on the genotype.

When comparing these findings obtained from patients with severe IGHD with the previous studies focusing on GHR allele genotype and response to rhGH replacement therapy, the patients, individual conditions, their related growth disorder, as well as the rhGH doses used have to be carefully analyzed (12, 14, 16). In the first report, for instance, Dos Santos *et al.* (12) studied patients with either SGA or ISS. In this study, patients with normal GH secretion were treated with supraphysiological rhGH replacement doses (12). Besides the various conditions studied, it is also possible that the differences between the studies reported so far represent the problems of sample size. False-positive findings are more likely with small samples sizes and for quantitative trait loci phenotypical variations tend to be overestimated with small sample sizes (32, 33). Only large-scale studies of well-defined conditions or pooling data sets will help to resolve these statistical issues. Therefore, it is worth pointing out that our sample size is to our knowledge among the largest reported to date.

Focusing on patients with GHD, Jorge *et al.* (13) pooled the data obtained from the patients with GHR-d3/d3 and d3/fl and compared their HV with that of patients carrying the GHR-fl/fl genotype. The conclusion drawn from the data were that patients with GHD who were homozygous for GHR-fl responded less well to the rhGH replacement. In addition, in this study 44 GH-deficient patients were also followed up to final height, of whom 22 carried the GHR-fl/fl and the remaining 22 either the d3/d3 or d3/fl genotype. A greater final height was achieved in patients with one or two copies of the GHR-d3 genotype when compared with the patients who were homozygous for the GHR-fl alleles (height SDS -0.8 *vs.* -1.7, respectively). In contrast to our study, in the study of Jorge *et al.* (13), not only subjects with IGHD but also combined pituitary hormonal deficiencies were analyzed, and, furthermore, HV data were only presented in prepubertal patients during the first year of treatment. This might at least partly explain the differences obtained. On the contrary, Pilotta *et al.* (15) could not detect an influence of GHR polymorphisms in the growth response of 54 prepubertal, rhGH-treated children. However, in comparison to our patients, these children had a higher maximal GH increase after stimulation tests (5 ± 2.9 ng/ml). Unfortunately, the authors did not show data analyzing the growth response according to the exon-3 genotype present (15). In addition, although in the report by Blum *et al.* (18) the GHR-d3 allele did not affect responsiveness to conventional rhGH replacement therapy, a statistical difference in IGF-I between the genotype groups fl/fl *vs.* d3/fl and d3/d3 was described. However, this finding could not be reproduced in our study but may underline the notion that responsiveness to GH treatment depends on the functional properties of the GHR. In the original study by Dos Santos *et al.* (12), HEK293 fibroblasts were transiently cotransfected with GHR-fl, GHR-d3, or both, and in these experiments, the GHR-d3 induced a higher transcriptional activity of the lactogenic hormone responsive element-luciferase reporter plasmid, which is activated by GH via binding to its GHR. In this study the dose response of GH was studied up to the dose of 50 ng/ml, and the effect in terms of lactogenic hormone responsive element-luciferase activation was described as linear. This increased, and allele dose-dependent responsiveness of the GHR-d3 to rhGH may explain the better growth response during the first years of rhGH treatment in subjects treated with the lower rhGH dose (26.5 *vs.* 36.5 μg/kg·d) as demonstrated using the multiple linear regression models in our study.

Obviously, neither GH responsiveness nor GH sensitivity is constant; the dose-response relationship may vary in every specific condition with/without an underlying growth disorder. Even for a specific condition and dose-response curve, an increase of rhGH dose may well itself affect GHR sensitivity, signaling, and, thus, response to treatment, as is well established in any rhGH-treated child whose response changes after the first years on treatment (2–8). Moreover, the dose response of rhGH

differs according to the condition that is treated. For instance, children with GHD, Turner syndrome, SGA, or ISS respond differently (and the rhGH dose is adapted accordingly), but a change in GH sensitivity with treatment remains a variable. Thus, the positive effect resulting from the GHR-d3 genotype may well be down-regulated and/or altered when supraphysiological doses of rhGH are given. This hypothesis could explain why severe GH-deficient subjects might present a rhGH dose-dependent GHR genotype-related effect on growth response with an apparent plateau at around 26 μg/kg·d (16 IU/m^2·wk), whereas this effect disappears with higher rhGH doses that lie further up on the dose-response curve for this condition. Similarly, SGA children treated with higher doses of rhGH showed no difference in growth response according to the exon-3 genotype in contrast to SGA children treated with lower doses (12, 16). Duration of GH treatment also seems to play a major role in defining the sensitivity to the GHR genotype, in addition to the etiology of the short stature and its severity.

Furthermore, as mentioned by Audi *et al.* (17), the GHR-d3/GHR-fl genotypes may well contribute to the wide range of growth in the normal population. Therefore, we studied 211 healthy Swiss control subjects of both Caucasian original and normal stature (17). Although the prevalence of the GHR genotypes obtained in our study differs from the Spanish controls, it is of importance, however, to highlight the identical finding that the prevalence of the GHR-d3/d3 and GHR-fl/fl genotypes in the taller subgroup (height of ≤ 2 and > 1 SDS) are respectively higher (13.1 *vs.* 7.4 and 8%) and lower (39.5 *vs.* 50 and 48%) when compared with the other two subgroups (Table 3). Similar observations were made when the genotype frequencies of the 248 parents were studied.

In summary, in our two studies focusing on patients with severe IGHD, we observed a GHR-d3 allele dose-dependent effect in subjects treated with rhGH during the first 2 yr, with significantly better responses of either the GHR d3/d3 or the GHR-d3/fl genotype, although no difference was observed at final height. Considering all the studies focusing on IGHD (13, 15, 18, 19), it becomes clear that the final impact of the GHR genotypes on the rhGH response is minimal. Given the importance of the response to attainment of final height, it may, nevertheless, well be an additional variable having some impact on growth and GH sensitivity, at least at the beginning of rhGH treatment.

Acknowledgments

We thank Professors Alex Odermatt and Markus Mohaupt, Division of Nephrology/Hypertonia, University Bern, Bern, Switzerland, for sharing DNA of the normal control population with us. We also thank Professor Iain Robinson for his great support and help during this work.

Address all correspondence and requests for reprints to: Professor Dr. Primus E. Mullis, University Children's Hospital, Pediatric Endocrinology, Diabetology, Metabolism, University of Bern, CH-3010 Bern, Switzerland. E-mail: primus.mullis@insel.ch.

This work was supported by Swiss National Research Foundation Grant 3200B0-105853/1 (to P.E.M.). P.E.M. is supported by the European Society for Pediatric Endocrinology Sabbatical leave Programe allowing to work at the National Institute of Medical Research at Mill Hill, London, United Kingdom, and at the London Centre for Pediatric Endocrinology and Metabolism, Institute of Child Health, London University College London, United Kingdom.

Disclosure Statement: The authors have nothing to disclose.

References

1. **Blethen SL, Baptista J, Kuntze J, Foley T, LaFranchi S, Johanson A** 1997 Adult height in growth hormone (GH)-deficient children treated with biosynthetic GH. The Genentech Growth Study Group. J Clin Endocrinol Metab 82:418–420
2. **Ranke MB, Lindberg A, Chatelain P, Wilton P, Cutfield W, Albertsson-Wikland K, Price DA** 1999 Derivation and validation of a mathematical model for predicting the response to exogenous recombinant human growth hormone (GH) in prepubertal children with idiopathic GH deficiency. KIGS International Board. Kabi Pharmacia International Growth Study. J Clin Endocrinol Metab 84:1174–1183
3. **Ranke MB, Lindberg A, Martin DD, Bakker B, Wilton P, Albertsson-Wikland K, Cowell CT, Price DA, Reiter EO** 2003 The mathematical model for total pubertal growth in idiopathic growth hormone (GH) deficiency suggests a moderate role of GH dose. J Clin Endocrinol Metab 88:4748–4753
4. **Ranke MB, Lindberg A, Cowell CT, Wikland KA, Reiter EO, Wilton P, Price DA, KIGS International Board** 2003 Prediction of response to growth hormone treatment in short children born small for gestational age: analysis of data from KIGS (Pharmacia International Growth Database). J Clin Endocrinol Metab 88:125–131
5. **Ranke MB, Cutfield WS, Lindberg A, Cowell CT, Albertsson-Wikland K, Reiter EO, Wilton P, Price DA, KIGS International Board** 2002 A growth prediction model for short children born small for gestational age. J Pediatr Endocrinol Metab 15(Suppl 5):1273
6. **Ranke MB, Lindberg A, Chatelain P, Wilton P, Price DA, Albertsson-Wikland K, KIGS International Board** 2001 The potential of prediction models based on data from KIGS as tools to measure responsiveness to growth hormone. Pharmacia International Growth Database. Horm Res 55(Suppl 2):44–48
7. **Ranke MB** 2000 New paradigms for growth hormone treatment in the 21st century: prediction models. J Pediatr Endocrinol Metab 13(Suppl 6):1365–1369
8. **Ranke MB, Lindberg A, Chatelain P, Wilton P, Cutfield W, Albertsson-Wikland K, Price DA, KIGS International Board** 2000 Kabi International Growth Study. Prediction of long-term response to recombinant human growth hormone in Turner syndrome: development and validation of mathematical models. KIGS International Board. Kabi International Growth Study. J Clin Endocrinol Metab 85:4212–4218
9. **Rosenfeld RG** 2006 Editorial: the pharmacogenomics of human growth. J Clin Endocrinol Metab 91:795–796
10. **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
11. **Pantel J, Grulich-Henn J, Bettendorf M, Strasburger CJ, Heinrich U, Amselem S** 2003 Heterozygous nonsense mutation in exon 3 of the growth hormone receptor (GHR) in severe GH insensitivity (Laron syndrome) and the issue of the origin and function of the GHRd3 isoform. J Clin Endocrinol Metab 88: 1705–1710
12. **Dos Santos C, Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P** 2004 A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 36:720–724
13. **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
14. **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
15. **Pilotta A, Mella P, Filisetti M, Felappi B, Prandi E, Parrinello G, Notarangelo, LD, Buzi F** 2006 Common polymorphisms of the growth hormone (GH) receptor do not correlate with the growth response to exogenous recombinant human GH in GH-deficient children. J Clin Endocrinol Metab 91:1178–1180
16. **Carrascosa A, Esteban C, Espadero R, Fernandez-Cancio M, Andaluz P, Clemente M, Audi L, Wollmann H, Fryklund L, Parodi L, Spanish SGA Study Group** 2006 The d3/fl-growth hormone (GH) receptor polymorphism does not in-

fluence 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
17. **Audi L, Esteban C, Carrascosa A, Espadero R, Perez-Arroyo A, Arjona R, Clemente M, Wollmann H, Fryklund L, Parodi LA, Spanish SGA Study Group** 2006 Exon 3-deleted/full-length growth hormone receptor polymorphism genotype frequencies in Spanish short small-for-gestational-age (SGA) children and adolescents (n = 247) and in an adult control population (n = 289) show increased fl/fl in short SGA. J Clin Endocrinol Metab 91:5038–5043
18. **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
19. **Ito Y, Makita Y, Matsuo K, Suzuki S, Ueda O, Mukai T, Tajima T, Fujieda K** 2006 Influence of the exon 3 deleted isoform of GH receptor gene on growth response to GH in Japanese children. Horm Res 65(Suppl 1):45
20. **Prader A, Largo RH, Molinari L, Issler C** 1989 Physical growth of Swiss children from birth to 20 years of age. Helv Paediatr Acta Suppl 52:1–125
21. **Brook CG** 1993 Growth. In: Brook CGD, ed. A guide to the practice of paediatric endocrinology. Cambridge, UK: Cambridge University Press; 18–53
22. **Marshall WA, Tanner JM** 1970 Variations in pattern of pubertal changes in boys. Arch Dis Child 45:15–23
23. **Ranke MB** 2003 Diagnosis of growth hormone deficiency and growth hormone stimulation tests. In: Ranke MB, ed. Diagnosis of endocrine function in children and adolescents. 3rd ed. Basel: Krager; 107–128
24. **Greulich WW, Pyle SI** 1959 Radiographic atlas of skeletal development of the hand and wrist. Stanford, CA: Stanford University Press
25. **Tanner JM, Whitehouse RH, Marshall WA, Carter BS** 1975 Prediction of adult height from height, bone age, and occurrence of menarche, at ages 4 to 16 with allowance for midparent height. Arch Dis Child 50:14–26
26. **Mullis PE, Robinson IC, Salemi S, Eble A, Besson A, Vuissoz JM, Deladoey J, Simon D, Czernichow P, Binder G** 2005 Isolated autosomal dominant growth hormone deficiency: an evolving pituitary deficit? A multicenter follow-up study. J Clin Endocrinol Metab 90:2089–2096
27. **Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB** 1993 Serum levels of insulin-like growth factor I (IGF-I) and IGF binding protein 3 reflect spontaneous growth hormone secretion. J Clin Endocrinol Metab 76:1610–1616
28. **Mullis PE, Akinci A, Kanaka C, Eble A, Brook CG** 1992 Prevalence of human growth hormone-1 gene deletions among patients with isolated growth hormone deficiency from different populations. Pediatr Res 31:532–534
29. **Chen EY, Liao YC, Smith DH, Barrera-Saldana HA, Gelinas RE, Seeburg PH** 1989 The human growth hormone locus: nucleotide sequence, biology, and evolution. Genomics 4:479–497
30. **Wagner JK, Eble A, Hindmarsh PC, Mullis PE** 1998 Prevalence of human GH-1 gene alterations in patients with isolated growth hormone deficiency. Pediatr Res 1998 43:105–110
31. **Reiter EO, Price DA, Wilton P, Albertsson-Wikland K, Ranke MB** 2006 Effect of growth hormone (GH) treatment on the near-final height of 1258 patients with idiopathic GH deficiency: analysis of a large international database. J Clin Endocrinol Metab 91:2047–2054
32. **Xu S** 2003 Theoretical basis of the Beavis effect. Genetics 165:2259–2268
33. **Beavis WD** 1998 QTL analyses: power, precision and accuracy. In: Paterson AH, ed. Molecular dissection of complex traits. Boca Raton, FL: CRC Press; 145–161