The Interactive Effect of *GHR-Exon 3* and −*202 A/C IGFBP3* Polymorphisms on rhGH Responsiveness and Treatment Outcomes in Patients with Turner Syndrome

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Context: There is great interindividual variability in the response to recombinant human (rh) GH therapy in patients with Turner syndrome (TS). Ascertaining genetic factors can improve the accuracy of growth response predictions.

Objective: The objective of the study was to assess the individual and combined influence of *GHR-exon 3* and −*202 A/C IGFBP3* polymorphisms on the short- and long-term outcomes of rhGH therapy in patients with TS.

Design and Patients: *GHR-exon 3* and −*202 A/C IGFBP3* genotyping (rs2854744) was correlated with height data of 112 patients with TS who remained prepubertal during the first year of rhGH therapy and 65 patients who reached adult height after 5 ± 2.5 yr of rhGH treatment.

Main Outcome Measures: First-year growth velocity and adult height were measured.

Results: Patients carrying at least one *GHR-d3* or −*202 A-IGFBP3* allele presented higher mean first-year growth velocity and achieved taller adult heights than those homozygous for *GHR-fl* or −*202 C-IGFBP3* alleles, respectively. The combined analysis of *GHR-exon* 3 and −*202 A/C IGFBP3* genotypes showed a clear nonadditive epistatic influence on adult height of patients with TS treated with rhGH (*GHR-exon* 3 alone, $R^2 = 0.27$; −*202 A/C IGFBP3*, $R^2 = 0.24$; the combined genotypes, $R^2 = 0.37$ at multiple linear regression). Together with clinical factors, these genotypes accounted for 61% of the variability in adult height of patients with TS after rhGH therapy.

Conclusion: Homozygosity for the *GHR-exon3* full-length allele and/or the −*202C-IGFBP3* allele are associated with less favorable short- and long-term growth outcomes after rhGH treatment in patients with TS. (*J Clin Endocrinol Metab* **97: E671–E677, 2012**)

Abbreviations: BA, Bone age; BMI, body mass index; CI, confidence interval; fl, full length; GHD, GH deficient; *GHR*, growth hormone receptor; *GHRd3, GHR*, exon 3 deleted; *GHRfl, GHR* fl isoform; IGFBP, IGF binding protein; rh, recombinant human; SDS, sd score; SNP, single-nucleotide polymorphism; TS, Turner syndrome.

Recombinant human (rh) GH is an approved therapy to improve final height of patients with Turner syndrome (TS), a common cause of short stature (1). Typically, patients with TS exhibit considerable interindividual variability regarding short- and long-term growth responses to rhGH therapy (2–5). Prediction models of responses to rhGH based on clinical parameters had limited capacity to explain this variability in TS as well as in other rhGH-treated growth disorders (6), suggesting that further parameters, such as genetic factors, may be missing from current models (4).

A meta-analysis has demonstrated that GH-deficient (GHD) and non-GHD children homozygous or heterozygous for the growth hormone receptor (*GHR*) exon 3 deleted (*GHRd3*) isoform had slightly better short-term growth outcomes after rhGH therapy than children homozygous for the full-length (fl) *GHR* isoform (*GHRfl*) (7). Three studies analyzed this polymorphism in patients with TS concerning the first-year growth response to rhGH treatment, without reproducible results (8–10), whereas only one study analyzed the influence of the *GHR*-exon 3 deleted isoform on final height (8). Additionally, a single-nucleotide polymorphism (SNP) located at position −202 of the IGF binding protein (IGFBP)-3 promoter region (rs2854744) has also been involved in rhGH pharmacogenetics of growth hormone deficiency (11) and children born small for gestational age (12).

In comparison with other non-GHD indications of rhGH therapy, TS is a more homogeneous cause of growth impairment and, consequently, constitutes a better model to test the influence of genetic variants on the rhGH growth response. Thus, the aim of this study was to assess the individual and combined influence of *GHR-exon 3* and −*202 A/C IGFBP3* polymorphisms on the short- and long-term outcomes of rhGH therapy in a large group of patients with TS.

Patients and Methods

Subjects

The study protocol was approved by the Hospital Ethics Committee, and informed consent was obtained from all patients or their parents before starting the molecular studies. One hundred twelve patients with TS were selected using the following criteria: 1) the presence of a karyotype containing a missing or a structurally aberrant X chromosome; 2) rhGH treatment on a daily schedule, and 3) patients remaining prepubertal throughout the first year of therapy. First-year growth velocity was determined after an observation period of 9–15 months. In addition, adult height was analyzed after 5 ± 2.5 yr of rhGH treatment in 65 patients. Adult height was defined by a documented growth velocity less than 1 cm/yr during the last 12 months. Patients were evaluated at baseline and every 4 months

during rhGH treatment. Evaluations were performed at the same period of the day and included measurements of weight (measured with a digital scale), standing height (mean of three measurements on a stadiometer) expressed in centimeters, and SD scores (SDS) for sex and age (13). Body mass index (BMI) was calculated (weight/height^2) and expressed as SDS (14). Target height was calculated [(father's height + mother's height − 13 cm)/2] and expressed as SDS. Left hand and wrist x-rays for bone age (BA) determination was assessed by the method of Greulich and Pyle (15).

Hormone assays

IGF-I levels were obtained at the start of treatment and near the end of the first year of rhGH therapy in 80% of the patients. IGF-I was measured by RIA after ethanol extraction (Diagnostic Systems Laboratories, Webster, TX) (73% of patients) or by chemiluminescence assays (IMMULITE; Diagnostic Products Corp., Los Angeles, CA) (27% of patients). IGFBP-3 levels were obtained at the start of treatment and near the end of the first year of rhGH therapy in 60% of the patients. IGFBP-3 was measured by immunoradiometric assay (Diagnostic Systems Laboratories) (66% of the patients) or by chemiluminescence assays (IMMULITE) (34% of the patients). IGF-I and IGFBP-3 levels were expressed as SDS for age and sex according to reference values provided by the respective assay kits.

Molecular studies

Genomic DNA was isolated from peripheral blood leukocytes by standard methods from all patients. The frequency of *GHR* transcript variants regarding the presence (*GHR*fl) or absence (*GHR*d3) of exon 3 was tested in all patients using a previously described multiplex PCR assay (16, 17). The polymorphism −202 A/C IGFBP3 (rs2854744) was genotyped by allelic discrimination in a Real Time 7500 system (Applied Biosystems, Foster City, CA) equipment using specific probes and primers (assay identification 186389191-1, TaqMan SNP genotyping assay; Applied Biosystems) according to the manufacturer's instructions. Ten percent of all samples were randomly regenotyped for quality control. The agreement of the genotypes determined by the blinded quality control samples was 100%.

Statistical analysis

Qualitative variables are listed as frequencies and percentages, whereas quantitative variables are shown as mean $\pm$ SD. Patients were compared by genotype relative to clinical and hormonal characteristics. The short-term response to rhGH was evaluated by growth velocity in the first year of treatment. The long-term response to rhGH was assessed by adult height SDS and adult height SDS adjusted for target height SDS. One-way ANOVA followed by a Tukey test was used for comparisons according to the additive model, whereas the t test was used for comparisons according to the dominant model. Numerical variables that did not demonstrate parametric distribution were analyzed by Kruskal-Wallis one-way ANOVA on ranks or Mann-Whitney rank sum test. Nominal variables were compared by a χ^2 or Fisher exact test, as appropriate. To assess whether genetic variants had independent prognostic significance for outcome, we performed single- followed by multiple-regression analyses adjusting for the established influential factors. The evaluated clinical factors included karyotype, birth length and weight, chronological age, BA, height, BMI at the start of treatment, target height, maternal and paternal height, induced or sponta-

TABLE 1. Clinical and hormonal characteristics of 112 patients with TS grouped according to *GHR*-exon 3 genotype

	GHR-exon 3 genotype				*P*	
	d3/d3	fl/d3	fl/fl	d3/*	d3/d3 *vs.* fl/d3 *vs.* fl/fl[a]	d3/* *vs.* fl/fl[b]
Number of patients for short-term outcomes	10	42	60	52		
Karyotype 45,X	40%	54%	50%	50%		
Target height SDS	−0.5 ± 0.8	−0.6 ± 0.8	−0.9 ± 0.8	−0.6 ± 0.8	ns	ns
Chronological age at the start of therapy (yr)	12 ± 3.2	11 ± 3.6	12 ± 3.9	11 ± 3.5	ns	ns
BA at the start of therapy (yr)	10 ± 2.7	9 ± 3.1	9 ± 3.1	9 ± 3.1	ns	ns
Height SDS at the start of therapy	−3.0 ± 0.7	−3.0 ± 1.0	−3.5 ± 0.8	−3.0 ± 0.9	0.012[c]	0.002
BMI SDS at the start of therapy	0.8 ± 0.6	0.5 ± 1.2	0.6 ± 1.1	0.5 ± 1.1	ns	ns
Mean rhGH dose (μg/kg · d)	48	48	48	48	ns	ns
First year growth velocity (cm/y)	7.8 ± 1.0	7.7 ± 1.7	6.3 ± 2.0	7.7 ± 1.6	<0.001[d]	<0.001
IGF-I SDS before rhGH therapy	−0.5 ± 1.4	−0.1 ± 1.6	−0.6 ± 1.2	−0.1 ± 1.6	ns	ns
IGF-I SDS after first year of rhGH therapy	1.5 ± 1.5	1.9 ± 1.4	1.4 ± 1.7	1.8 ± 2.0	ns	ns
IGFBP-3 SDS before rhGH therapy	−0.4 ± 1.0	0.2 ± 1.5	−0.2 ± 1.1	0.2 ± 1.4	ns	ns
IGFBP-3 SDS after first year of rhGH therapy	0.7 ± 1.0	1.0 ± 1.5	0.6 ± 1.0	1.0 ± 1.4	ns	ns
Number of patients for long-term outcomes	9	22	34	31		
Spontaneous:induced puberty	1:8	5:17	4:30	6:25	ns	ns
Chronological age at onset of puberty (yr)	14 ± 2.4	14 ± 1.7	15 ± 2.7	14 ± 1.9	ns	ns
rhGH therapy before onset of puberty (yr)	3.0 ± 2.0	4.0 ± 2.4	4.0 ± 2.8	4.0 ± 2.6	ns	ns
Duration of rhGH therapy (yr)	4.0 ± 0.9	5.1 ± 2.6	5 ± 2.4	4.8 ± 2.4	ns	ns
Adult height SDS	−1.2 ± 0.9	−1.4 ± 0.6	−2.5 ± 1.0	−1.4 ± 0.7	<0.001[e]	<0.001
Adult height-target height SDS	−0.7 ± 1.3	−0.8 ± 0.7	−1.4 ± 1.2	−0.7 ± 0.9	ns	0.01
Height gain SDS	1.6 ± 1.2	1.1 ± 1.2	0.7 ± 1.5	1.2 ± 1.2	ns	ns

d3/* indicates combined fl/d3 and d3/d3-*GHR* genotypes. ns, Not significant.

[a] One-way ANOVA.

[b] *t* test.

[c] Tukey test: d3/d3 *vs.* fl/fl = ns; d3/d3 *vs.* fl/d3 = ns; fl/d3 *vs.* fl/fl = ns.

[d] Tukey test: d3/d3 *vs.* fl/fl = $P < 0.05$; d3/d3 *vs.* fl/d3 = ns; fl/d3 *vs.* fl/fl = $P < 0.05$.

[e] Tukey test: d3/d3 *vs.* fl/fl = $P < 0.05$; d3/d3 *vs.* fl/d3 = ns; fl/d3 *vs.* fl/fl = $P < 0.05$.

neous puberty, age and height at the start of puberty, rhGH doses, and duration of treatment. A $P < 0.05$ was considered statistically significant. All statistical analyses were performed by SigmaStat for Windows (version 3.5; SPSS, Inc., San Rafael, CA).

Results

Patients' characteristics and genotyping distribution

At the start of rhGH therapy with a mean dose of 48 μg/kg · d, the patients with TS (n = 112) had a chronological age of 11.2 ± 3.8 yr, bone age delay (mean BA of 9.0 ± 3.1 yr), and a mean height SDS of −3.2 ± 1.1. The onset of puberty, induced in 92 and spontaneous in 20 patients, was at 14.3 ± 2.4 yr, and 65 patients achieved adult height (mean height SDS of −2.0 ± 1.0) after 5.0 ± 2.5 yr of rhGH treatment.

All patients were genotyped for *GHR-exon 3* [genotype distribution: 53.6% (fl/fl); 37.5% (fl/d3); and 8.9% (d3/d3)] and −*202 A/C IGFBP3* polymorphism [genotype distribution: 17% (A/A); 48.2% (A/C); and 34.8% (C/C)]. The genotype distributions were in Hardy-Weinberg equilibrium. Genotypic groups were similar concerning karyotype distribution, chronological and bone ages at the start

of treatment, basal BMI, parental height, the frequency of spontaneous or induced puberty, age at puberty onset, mean rhGH dose, and duration of therapy (Tables 1 and 2). However, patients homozygous for *GHR*-fl allele and for −202 C-*IGFBP3* allele had lower basal height SDS than patients carrying *GHR-d3* and −*202 A-IGFBP-3* alleles, respectively, at the start of therapy (Tables 1 and 2). Patients homozygous for the −202 C-*IGFBP3* allele also had IGFBP-3 levels on average −1.0 SDS lower [95% confidence interval (CI) −1.5 to −0.5; $P < 0.001$] than carriers of at least one −*202 A-IGFBP-3* allele.

Clinical correlations

Short-term growth response

Patients homozygous for the *GHR-fl* allele demonstrated lower growth velocity in the first year of rhGH treatment when compared with those patients with at least one *GHR-exon 3* deleted allele (Table 1). As a group, patients with the *GHR* d3/* genotype had growth velocity on average 1.4 cm/yr higher than those with *GHR* fl/fl genotype (95% CI 0.7–2.0 cm/yr; $P < 0.001$).

Similarly, patients homozygous for the −202 C-*IGFBP3* allele presented lower growth velocity in the first year of

TABLE 2. Clinical and hormonal features of 112 patients with TS grouped according to −202 A/C *IGFBP*3 genotype

	−202 A/C *IGFBP*3 genotype				P	
	A/A	**A/C**	**C/C**	**A/***	**A/A vs. A/C vs. C/C**[a]	**A/* vs. C/C**[b]
Number of patients for short-term outcomes	19	54	39	73		
Karyotype 45,X	57%	48%	56%	51%		
Target height SDS	−0.5 ± 0.6	−0.9 ± 0.8	−0,8 ± 0.9	−0.8 ± 0.8	ns	ns
Chronological age at the start of therapy (yr)	11 ± 3.5	11 ± 3.8	11 ± 3.9	11 ± 3.7	ns	ns
BA at the start of therapy (yr)	10 ± 2.3	9 ± 3.2	9 ± 3.2	9 ± 3.0	ns	ns
Height SDS at the start of therapy	−3.0 ± 0.9	−3.0 ± 1.1	−3.5 ± 1.3	−3.0 ± 1.1	ns	0.034
BMI SDS at the start of therapy	0.4 ± 0.8	0.6 ± 1.1	0.8 ± 1.1	0.6 ± 1.1	ns	ns
Mean rhGH dose (μg/kg · d)	48	48	48	48	ns	ns
First-year growth velocity (cm/yr)	7.4 ± 1.6	7.1 ± 1.9	6.4 ± 2.1	7.2 ± 1.8	ns	0.037
IGF-I SDS before rhGH therapy	−0.3 ± 1.1	−0.3 ± 1.5	−0.5 ± 1.5	−0.3 ± 1.4	ns	ns
IGF-I SDS after first year of rhGH therapy	1.3 ± 1.0	1.9 ± 2	1.2 ± 2	1.8 ± 1.8	ns	ns
IGFBP-3 SDS before rhGH therapy	0.2 ± 1.1	0.2 ± 1.5	−0.8 ± 1.0	0.2 ± 1.3	<0.001[c]	<0.001
IGFBP-3 SDS after first year of rhGH therapy	1.2 ± 0.5	0.9 ± 1.4	0.4 ± 1.0	0.9 ± 1.3	<0.030[d]	0.039
Number of patients for long-term outcomes	10	35	20	45		
Spontaneous:induced puberty	2:8	7:28	1:19	9:36	ns	ns
Chronological age at onset of puberty (yr)	15.0 ± 1.6	14.0 ± 2.3	15.0 ± 2.7	14.2 ± 2.2	ns	ns
rhGH therapy before onset of puberty (yr)	3.1 ± 2.0	4.0 ± 2.7	3.3 ± 2.0	4.0 ± 2.7	ns	ns
Duration of rhGH therapy (yr)	5.0 ± 2.3	5.0 ± 2.7	5.0 ± 2.4	5.0 ± 2.6	ns	ns
Adult height SDS	−1.0 ± 0.7	−1.9 ± 1.0	−2.7 ± 0.8	−1.7 ± 1.0	<0.001[e]	<0.001
Adult height–target height SDS	−0.5 ± 0.7	−1.0 ± 1.0	−1.6 ± 1.4	−0.9 ± 1.0	0.031[d]	0.025
Height gain SDS	1.7 ± 0.8	1.0 ± 1.2	0.5 ± 1.6	1.2 ± 1.1	ns	0.045

A/* indicates combined A/C and A/A −202-*IGFBP*3 genotypes. ns, Not significant.

[a] One-way ANOVA.

[b] Student *t* test.

[c] Tukey test: A/A *vs.* C/C = *P* < 0.05; A/A *vs.* A/C = ns; A/C *vs.* C/C = *P* < 0.05.

[d] Tukey test: A/A *vs.* C/C = *P* < 0.05; A/A *vs.* A/C = ns; A/C *vs.* C/C = ns.

[e] Tukey test: A/A *vs.* C/C = *P* < 0.05; A/A *vs.* A/C = *P* < 0.05; A/C *vs.* C/C = *P* < 0.05.

rhGH therapy when compared with those carrying at least one −202 A-*IGFBP3* allele (Table 2). The −202 A/* *IGBFP3* genotype group had a growth velocity on average 0.8 cm/yr higher than the −202 C/C-*IGFBP3* group (95% CI 0.05–1.6 cm/yr, *P* = 0.037). There was a significant relationship between −202 A/*-*IGFBP3* group and circulating IGFBP-3 levels at baseline (IGFBP-3 SDS 0.2 ± 1.3 for the −202 A/*-*IGFBP3* group and −0.8 ± 1.0 for the −202-C/C-*IGFBP3* group; *P* < 0.001) and after the first year of rhGH treatment (IGFBP-3 SDS 0.9 ± 1.3 for the −202 A/*-*IGFBP3* group and 0.4 ± 1.0 for the −202-C/C-*IGFBP3* group; *P* = 0.039).

Combined analysis showed that *GHR-exon 3* and −202 A/C *IGFBP3* genotypes had an interactive influence on the first year growth velocity (Table 3). Patients with TS presenting the combination of the two favorable genotypes (*GHR-exon 3* d3/* + −202 A/* *IGFBP3*) had first-year growth velocity on average 1.8 cm/yr (95% CI 1.0–2.6 cm/yr, *P* < 0.001) higher than individuals with unfavorable combined genotypes (*GHR-exon 3* fl/fl + −202 C/C *IGFBP3*), whereas patients with intermediate genotypes (fl/fl + A/* or d3* + C/C) had in-between values (Table 3).

Multiple linear regressions adjusting for other clinical variables showed that *GHR*-exon 3 genotype was an in-

dependent prediction variable for first-year growth velocity. Together with chronological age (*P* < 0.001) and basal BMI SDS (*P* = 0.018) at onset of rhGH therapy, *GHR*-exon 3 genotype (*P* = 0.001) explained 40% of first-year growth velocity variability. There was no significant increment on first-year growth velocity prediction after adding −202 A/C *IGFBP3* polymorphism (R^2 = 0.41 with and R^2 = 0.40 without the inclusion of −202 A/C *IGFBP3* genotypes).

Long-term growth response

The positive influence of the *GHR-d3* and −202 A-*IGFBP3* alleles noted on first-year growth velocity was also observed on adult height of patients with TS after rhGH therapy, with or without adjustment for target height (Tables 1 and 2). Patients carrying at least one *GHR-d3* allele were 1.1 SDS (95% CI 0.7–1.5, *P* < 0.001) taller than those homozygous for the *GHR-fl* allele (Table 1). Carriers of at least one −202 A-*IGFBP3* allele were 1.0 SDS (95% CI 0.5–1.5, *P* < 0.001) taller than those homozygous for the −202 C-*IGFBP3* allele (Table 2).

Combined analysis including *GHR-exon 3* and −202 A/C *IGFBP3* genotypes demonstrated that they had an independent and interactive influence on adult height. De-

TABLE 3. Clinical and hormonal features of 112 patients with TS grouped according to combined *GHR*-exon 3 and −202 *IGFBP3* genotypes

| | *GHR*-exon 3 and −202 *IGFBP3* genotypes | | | *P* |
	d3/*+ A/* (1)	fl/fl+A/* or d3*+C/C (2)	fl/fl+C/C (3)	(1) *vs.* (2) *vs.* (3)[a]
Number of patients for short-term outcomes	44	37	31	
Karyotype 45,X	50%	56%	49%	
Target height SDS	−0.6 ± 0.8	−0.8 ± 0.8	−1 ± 0.9	ns
Chronological age at the start of therapy (yr)	11 ± 3.6	11 ± 3.7	12 ± 4	ns
BA at the start of therapy (yr)	9 ± 3.1	9 ± 3.1	10 ± 3.1	ns
Height SDS at the start of therapy	−3.0 ± 0.9	−3.1 ± 1.2	−3.7 ± 1.3	0.023[b]
BMI SDS at the start of therapy	0.7 ± 1.1	0.4 ± 1.0	0.8 ± 1.2	ns
Mean rhGH dose (μg/kg · d)	48	48	48	ns
First-year growth velocity (cm/yr)	7.8 ± 1.6	6.8 ± 1.9	6.0 ± 2.0	<0.001[c]
IGF-I SDS before rhGH therapy	−0.1 ± 1.6	−0.3 ± 1.3	−0.8 ± 1.3	ns
IGF-I SDS after first year of rhGH therapy	1.9 ± 2.0	1.7 ± 1.6	1.1 ± 1.9	ns
IGFBP-3 SDS before rhGH therapy	0.2 ± 1.6	0.2 ± 1.0	−1.1 ± 0.8	<0.001[d]
IGFBP-3 SDS after first year of rhGH therapy	1.1 ± 0.9	0.6 ± 0.8	0.4 ± 1.1	0.004[c]
Number of patients for long-term outcomes	27	21	17	
Spontaneous:induced puberty	6:21	3:18	1:16	ns
Chronological age at onset of puberty (yr)	14.0 ± 1.9	14.5 ± 2.3	15.0 ± 2.8	ns
rhGH therapy before onset of puberty (yr)	4.0 ± 2.6	3.0 ± 2.0	3.0 ± 2.0	ns
Duration of rhGH therapy (yr)	5.0 ± 2.5	4.5 ± 2.4	5.0 ± 2.4	ns
Adult height SDS	−1.3 ± 0.7	−2.2 ± 1.8	−2.7 ± 0.8	<0.001[c]
Adult height-target height SDS	−0.7 ± 0.9	−1.4 ± 0.9	−1.6 ± 1.4	0.014[b]
Height gain SDS	1.3 ± 1.1	0.8 ± 1.3	0.6 ± 1.6	ns

ns, Not significant.

[a] One-way ANOVA.

[b] Tukey test: (1) *vs.* (3) = $P < 0.05$; (1) *vs.* (2) = ns; (2) *vs.* (3) = ns.

[c] Tukey test: (1) *vs.* (3) = $P < 0.05$; (1) *vs.* (2) = $P < 0.05$; (2) *vs.* (3) = ns.

[d] Tukey test: (1) *vs.* (3) = $P < 0.05$; (1) *vs.* (2) = ns; (2) *vs.* (3) = $P < 0.05$.

spite similar rhGH doses and duration of treatment, patients with TS who presented the combination of the two favorable genotypes (*GHR-exon 3* d3/* + −202 A/* *IGFBP3*) reached adult height on average 1.4 SD (95% CI of 0.9–1.9, $P < 0.001$) higher than those girls with TS carrying unfavorable combined genotypes (*GHR*-exon 3

FIG. 1. The absolute values of height (centimeters) (A) and number of patients (B) according to the *GHR exon3* and −202 *A/C IGFBP-3* genotypes.

fl/fl ± −202 C/C *IGFBP3*; $P < 0.001$). Patients with intermediate genotypes (fl/fl + A/* or d3* + C/C) had in-between results (Table 3 and Fig. 1).

Alone, the *GHR-exon 3* genotype accounted for 27% of adult height variation ($P < 0.001$), whereas the −202 *A/C IGFBP3* genotype accounted for 24% ($P < 0.001$).

Combined, these two variants were responsible for 37% of adult height variation ($P < 0.001$) and together with basal height SDS ($P < 0.001$) and chronological age at onset of puberty ($P < 0.001$) explained 61% of the observed variation.

Discussion

In recent years, rhGH pharmacogenetic studies demonstrated that common polymorphisms can modulate the response to rhGH treatment (11, 18–20). The addition of these genetic factors to established clinical variables might improve the accuracy of growth response prediction, allowing for individualized
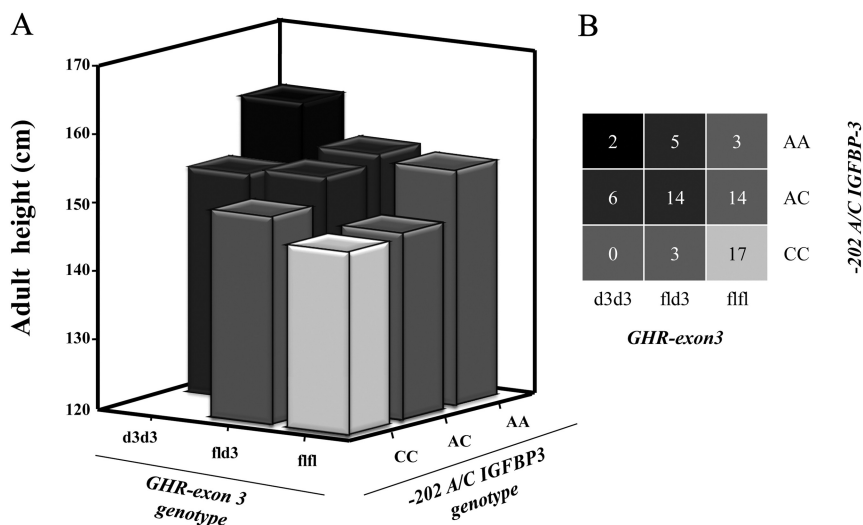
therapy. One of the limitations of rhGH pharmacogenetic studies is the significant variability in the etiology of short stature in some conditions in which rhGH therapy is used, such as idiopathic short stature and children born small for gestational age (SGA) (21, 22). TS, on the other hand, is a more homogeneous cause of short stature because *SHOX* gene haploinsufficiency is the main cause of growth impairment (23).

The present study confirmed the positive influence of *GHR-d3* allele on not only the first-year growth velocity (24) but also adult height outcomes (8) in patients with TS treated with rhGH. The absence of correlation between *GHR-exon* 3 genotype and growth response to rhGH in two previous studies in patients with TS can be partially explained by the small numbers of patients enrolled (9) and the paucity of individuals carrying the *GHR-d3* allele (10), precluding sufficient statistical power. A published meta-analysis concluded that the *GHR-d3* allele is associated with better growth response to rhGH in GHD and non-GHD children with short stature (7). Although alone the *GHR-exon 3* genotype accounts for a slight difference in growth response between genotype groups (7), its association with other polymorphisms might improve prediction models resulting in a better individualization of rhGH treatment. In the present study, for the first time, the combined impact of genotypes at different loci on the magnitude of growth response to rhGH therapy in the patients with Turner syndrome was analyzed.

IGFBP-3 is known to modulate the actions of IGF and also exhibits distinct biological effects independent of the IGF/IGF-I receptor axis (25–29). The −202 A/C SNP of the *IGFBP3* promoter region has been correlated with circulating IGFBP-3 levels in healthy adults (30), GHD children (11), and children born small for gestational age (12). In agreement with these findings, in the present study, patients with TS carrying the −202A-IGFBP3 allele also had mean IGFBP-3 levels higher than patients homozygous for the −202C-IGFBP3 allele. Additionally, the presence of the −202A-IGFBP3 allele was also associated with better short- and long-term growth outcomes, similar to what was observed for patients with severe GHD (11).

It is noteworthy that the combined analysis of the *GHR-exon* 3 and −202 A/C *IGFBP3* disclosed a clear nonadditive epistatic influence of these two common polymorphisms on adult height of patients with TS treated with rhGH (*GHR-exon* 3 alone, $R^2 = 0.27$; −202 A/C *IGFBP3*, $R^2 = 0.24$; the combined genotypes, $R^2 = 0.37$, Fig. 1). Together with the clinical factors, these genotypes accounted for 61% of variability in adult height of patients with TS after rhGH therapy.

We conclude that homozygosity for the *GHR-exon3* full-length allele and −202 C-IGFBP3 allele are associated with less favorable short- and long-term growth outcomes after rhGH treatment in patients with Turner syndrome. Furthermore, these polymorphisms exhibit a nonadditive interaction in rhGH outcomes. The combined influence of multiple as-yet-undiscovered genetic factors with the already known ones might improve the accuracy of the prediction of growth response allowing the desired personalized medicine. The idea of personalized treatment proposes that the combination of clinical and genetic factors could influence the treatment strategy in each individual patient, including the choice of drug dose. Our data support the idea that rhGH dose should be tailored at the start of treatment according to the worst/best clinical and genotypic profile. This concept needs to be proved in prospective studies. Patients with less favorable genotypes could benefit from higher rhGH doses improving height outcome. On the other hand, patients with the best responsive genotypes could benefit from dose reduction and consequently treatment cost and side effects without impairment in height outcome.

Acknowledgments

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