The −202 A Allele of Insulin-Like Growth Factor Binding Protein-3 (*IGFBP3*) Promoter Polymorphism Is Associated with Higher IGFBP-3 Serum Levels and Better Growth Response to Growth Hormone Treatment in Patients with Severe Growth Hormone Deficiency

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Context: Genetic factors that influence the response to recombinant human GH (rhGH) therapy remain mostly unknown. To date, only the GH receptor gene has been investigated.

Objective: The aim of the study was to assess the influence of a polymorphism in the IGF-binding protein-3 (IGFBP-3) promoter region (−202 A/C) on circulating IGFBP-3 levels and growth response to rhGH therapy in children with GH deficiency (GHD).

Design and Patients: −202 A/C *IGFBP3* genotyping (rs2854744) was correlated with data of 71 children with severe GHD who remained prepubertal during the first year of rhGH treatment.

Main Outcome Measures: We measured IGFBP-3 levels and first year growth velocity (GV) during rhGH treatment.

Results: Clinical and laboratory data at the start of treatment were indistinguishable among patients with different −202 A/C *IGFBP3* genotypes. Despite similar rhGH doses, patients homozygous for the A allele presented higher IGFBP-3 SD score levels and higher mean GV in the first year of rhGH treatment than patients with AC or CC genotypes (first year GV, AA = 13.0 ± 2.1 cm/yr, AC = 11.4 ± 2.5 cm/yr, and CC = 10.8 ± 1.9 cm/yr; $P = 0.016$). Multiple linear regression analyses demonstrated that the influence of −202 A/C *IGFBP3* genotype on IGFBP-3 levels and GV during the first year of rhGH treatment was independent of other variables.

Conclusion: The −202 A allele of *IGFBP3* promoter region is associated with increased IGFBP-3 levels and GV during rhGH treatment in prepubertal GHD children. **(*J Clin Endocrinol Metab* 94: 588–595, 2009)**

GH replacement is a standard therapy for children with short stature due to GH deficiency (GHD). The usual treatment is carried out with empirical and fixed doses of recombinant human GH (rhGH) adjusted uniquely for body weight or surface (1). Although it is expected that rhGH replacement completely resolves growth impairment in children with severe GHD, these patients exhibit considerable interindividual variability regarding growth responses to rhGH.

Some of the clinical factors that influence growth response to rhGH have already been identified, such as maximum GH peak

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Abbreviations: BMI, Body mass index; CLIA, chemiluminescence assay; CPHD, combined pituitary hormone deficiency; GHD, GH deficiency; IGFBP-3, IGF-binding protein-3; IGHD, isolated GHD; IFMA, immunofluorometric assay; IRMA, immunoradiometric assay; rhGH, recombinant human GH; SDS, SD score.

in provocative tests (2–5), age and height SD scores (SDS) at the beginning of treatment (2–4, 6), target height (3–5), and duration of treatment (3, 5, 6). However, these variables explain only 40 to 61% of rhGH responsiveness at the first year of therapy and 37 to 58% of variability at final height, implying that further parameters may be missing from current prediction models.

Until recently, genetic factors that influence growth response to rhGH were indirectly taken into account by including parental heights in the growth prediction models (3–5). To date, only the GH receptor gene has been investigated in rhGH pharmacogenetic studies (7–9). Despite the heterogeneity of early results, these studies introduced the important concept that common variations in GH/IGF-I axis genes may play a role in predicting response to rhGH therapy.

GH promotes growth mainly by IGF-I, which acts by endocrine and paracrine mechanisms. IGF-I transport in serum is mediated mostly by IGF-binding protein-3 (IGFBP-3) and acid labile subunit. Besides being the major carrying protein for IGF-I in the circulation, IGFBP-3 can act as a modulator of IGF-I bioactivity and has IGF-I-independent actions on growth regulation at the tissue level (10–14). Twin studies indicated that about 60% of the interindividual variability in circulating levels of IGFBP-3 is genetically determined (15). The *IGFBP3* gene is highly conserved among species and is present as a single copy on chromosome 7p14-p12. In 2001, Deal *et al.* (16) identified several single-nucleotide polymorphisms in the promoter region of *IGFBP3*. The most relevant single-nucleotide polymorphism (rs2854744) was an A to C nucleotide change located 202 bp upstream to the transcription start site, near elements believed to control basal promoter activity of *IGFBP3*. Genotype at this locus was highly correlated to circulating levels of IGFBP-3 in healthy adults. Mean IGFBP-3 levels were highest in individuals with the AA genotype and declined significantly in a stepwise manner in the presence of one or two copies of the C allele. Further studies confirmed this genotype-phenotype association (17–23). Additionally, *in vitro* studies documented significantly higher promoter activity of the A allele in comparison to the C allele (16), supporting the functional importance of −202 A/C polymorphism.

Until now, there were no data on the effect of this polymorphism on the IGFBP-3 serum levels and growth response to rhGH treatment in children with GHD. Therefore, the aim of our study was to evaluate the influence of the −202 A/C *IGFBP3* promoter polymorphism on the serum levels of IGFBP-3 and growth response to rhGH therapy in children with severe GHD.

Subjects and Methods

Subjects

Seventy-one severe GHD patients who were treated exclusively with rhGH on a daily schedule and who remained prepubertal throughout the first year of therapy were selected for evaluation of first year growth velocity and IGFBP-3 serum levels. Among these patients, 36 have already reached adult height after 8 ± 3 yr of rhGH treatment and were also analyzed regarding final height growth outcomes. GHD diagnosis was based on clinical and auxological parameters as well as the failure of GH response on at least two provocative tests. Patients with central nervous system tumors, meningoencephalocele, or previous radiation therapy were not included.

Magnetic resonance imaging of the hypothalamic-pituitary region was performed in all patients. According to the basal levels and hormonal response to the combined test (insulin + TRH + GnRH), patients were classified as isolated GHD (IGHD) or combined pituitary hormone deficiency (CPHD). Patients with CPHD were on regular replacement therapy for the other hormonal deficiencies.

The study protocol was approved by the Hospital Ethics Committee, and informed consent was obtained from all patients or their parents before initiating the molecular studies.

Study protocol

rhGH was administered sc at a mean dose of 32 μg/kg · d (0.1 IU/kg · d), which was adjusted according to changes in weight at each visit. All children were evaluated at baseline and every 3–4 months during rhGH treatment. First year growth velocity was determined after an observation period of at least 11 months. Among the 36 GHD patients who completed growth, the adult height was measured, on average, 1.4 yr after rhGH withdrawal. Evaluations were performed at the same period of day and included measurements of weight (measured with a digital scale) and standing height (measured with a stadiometer). Height and body mass index (BMI) were expressed as SDS (24, 25). Target height was calculated [(father's height + mother's height + 13 cm for boys or − 13 cm for girls)/2] and expressed as SDS. Left hand and wrist x-rays for bone age determination were assessed based on the method of Greulich and Pyle (26). IGF-I and IGFBP-3 levels were measured both before and during therapy.

Hormone assays

GH was initially measured by immunoradiometric assay (IRMA) (61% of the patients) and subsequently by immunofluorometric assay (IFMA) (AutoDELFIA, Wallac, Turku, Finland) method with monoclonal antibodies (39% of the patients). Cutoff levels used for GHD diagnosis were peak GH levels no greater than 5.0 μg/liter (IRMA) or no greater than 3.3 μg/liter (IFMA).

IGF-I levels were obtained at the start of treatment and near the end of the first year of rhGH therapy in 65 and 77% of patients, respectively. IGF-I was measured by RIA after ethanol extraction (Diagnostic Systems Laboratories, Webster, TX) (91% of patients) or by chemiluminescence assays (CLIA) (IMMULITE; Diagnostic Products Corp., Los Angeles, CA) (9% of patients). IGFBP-3 levels were obtained at the start of treatment and near the end of the first year of rhGH therapy in 51 and 72% of patients, respectively. IGFBP-3 was measured by IRMA (Diagnostic Systems Laboratories, 90%) or CLIA (IMMULITE, 10%). 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. A 376-bp fragment encompassing the −202 A/C polymorphic site at the promoter region of *IGFBP3* gene was amplified using specific primers, and PCR products were digested with *Fsp*I enzyme (New England Biolabs, Beverly, MA). In the presence of the C allele, the 376-bp PCR fragment is digested into 260-bp and 116-bp fragments, whereas in the presence of the A allele, the restriction site is absent. Primer sequences, amplification protocols, and digestion protocols will be sent upon request.

The validity of the PCR-restriction-fragments length polymorphism analysis was verified by direct sequencing of each genotype using the BigDye FN Sequencing kit (PE Applied Biosystems, Foster City, CA). For quality control, we regenotyped approximately 10% of random samples. The agreement of the genotypes determined for the blinded quality control samples was 100%.

Statistical analysis

Qualitative variables are listed as frequencies and percentages, whereas quantitative variables are shown as mean ± SD. Patients were compared by genotype relative to clinical and laboratory 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 minus target height SDS. IGF-I and IGFBP-3 levels were evaluated as SDS for age and sex both before and during treatment.

Genotype-group comparisons were performed according to two genetic models: a codominant model, in which the three genotypes were analyzed separately (AA *vs.* AC *vs.* CC), and a recessive model, in which patients homozygous for the A allele (AA group) were compared with patients presenting at least one copy of the C allele (AC + CC group). The recessive model was the most frequent approach adopted in previous studies, which evaluated the effects of −202 A/C *IGFBP3* polymorphism (16, 17, 20–22). ANOVA followed by Tukey test was used for comparisons according to the codominant model (AA *vs.* AC *vs.* CC), whereas the *t* test was used for comparisons according to the recessive model (AA *vs.* AC + CC). Numerical variables that did not demonstrate parametric distribution (GH peak levels and mean rhGH doses) were analyzed by Kruskal-Wallis one-way ANOVA on ranks or Mann-Whitney rank sum test. Nominal variables were compared by 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. A P value <0.05 was considered statistically significant. All statistical analyses were performed with SigmaStat for Windows (version 2.03; SPSS, Inc., San Rafael, CA).

Results

Patient's phenotype at the start of therapy

Seventy-one prepubertal GHD children (48 boys and 23 girls) were evaluated. At the start of treatment, they presented a chronological age of 8.6 ± 4.1 yr, with marked bone age delay (4.3 ± 2.7 yr) and short stature (height SDS, −4.3 ± 1.4). Severe GHD was demonstrated by extremely low GH peak levels, obtained at two different stimulation tests (mean GH peak, 0.9 ± 0.9 μg/liter; range, <0.1 to 3.3 μg/liter). No difference in mean GH peak was observed between patients diagnosed by IFMA or IRMA GH assays. Ninety-two percent of patients had either a defined genetic etiology for GHD (12 patients) or an anatomic abnormality of the pituitary gland on magnetic resonance imaging (ectopic posterior lobe in 45 patients and interrupted stalk in 18). Twenty-nine patients presented IGHD, and 42 had CPHD. Among the latter, central hypothyroidism was found in 77%, ACTH deficiency in 45%, and diabetes insipidus in 12%; in 53%, central hypogonadism was diagnosed during follow-up. Patients with IGHD and CPHD presented similar growth responses during rhGH treatment and, therefore, were analyzed together. Pretreatment growth velocity was not available because, due to the severity of short stature in the majority of patients, treatment was started after prompt GHD diagnosis.

Clinical correlations with −202 A/C *IGFBP3* genotyping

The distribution of patients among the different −202 *IGFBP3* genotypes was 21% AA, 54% AC, and 25% CC. The genotype frequencies conformed to the Hardy-Weinberg equilibrium test. There were no significant differences between genotype groups regarding clinical features at the start of treatment, GH peak at stimulation tests, and mean rhGH dose during treatment (Tables 1 and 2).

IGF-I and IGFBP-3 SDS levels

Before the start of treatment, there was a tendency to lower IGFBP-3 levels adjusted for age and sex in patients presenting one or two copies of the −202 C *IGFBP3* allele, but this difference was not significant. During rhGH treatment, patients homozygous for the A allele presented significantly higher IGFBP-3 levels than C allele carriers in codominant ($P = 0.002$) and recessive models ($P < 0.001$) (Table 1 and Fig. 1). Additionally, patients homozygous for the A allele presented significant higher incre-

TABLE 1. Clinical and auxological characteristics of 71 children with GHD grouped according to −202 A/C *IGFBP3* genotype

	−202 A/C *IGFBP3* genotype				*P*	
	AA	AC	CC	AC + CC	*AA vs. AC vs. CC*	*AA vs. AC + CC*
n	15	38	18	56		
Gender (males:females)	11:4	26:12	11:7	37:19	ns	ns
IGHD:CPHD	14:11	17:21	8:10	25:31	ns	ns
Target height SDS	−1.1 ± 0.9	−0.8 ± 0.8	−1.0 ± 0.8	−0.9 ± 0.8	ns	ns
Highest GH peak (μg/liter)	0.5 ± 0.7	0.9 ± 0.8	1.0 ± 1.0	1.0 ± 0.9	ns	ns
Chronological age (yr)	9.6 ± 4.8	8.5 ± 4.3	8.2 ± 3.1	8.4 ± 3.9	ns	ns
Bone age delay (yr)	5.2 ± 3.1	4.2 ± 2.8	3.8 ± 2.1	4.1 ± 2.6	ns	ns
Basal height SDS	−4.2 ± 1.2	−4.5 ± 1.6	−3.7 ± 0.7	−4.3 ± 1.4	ns	ns
Basal BMI SDS	−0.6 ± 1.3	−0.5 ± 1.3	0.0 ± 1.3	−0.3 ± 1.3	ns	ns
Mean rhGH dose (μg/kg · d)	29 ± 9	32 ± 10	29 ± 9	32 ± 8	ns	ns
IGFBP-3 SDS pretreatment	−2.3 ± 0.9	−2.6 ± 1.3	−2.9 ± 0.5	−2.7 ± 1.1	ns	ns
IGFBP-3 SDS during treatment	0.4 ± 1.9	−1.0 ± 1.0	−1.5 ± 1.4	−1.2 ± 1.2	0.002[a]	<0.001
IGF-I SDS pretreatment	−1.8 ± 1.4	−2.4 ± 1.6	−2.3 ± 1.5	−2.3 ± 1.5	ns	ns
IGF-I SDS during treatment	−0.5 ± 1.6	−0.1 ± 1.9	−0.8 ± 0.9	−0.3 ± 1.7	ns	ns
First year growth velocity (cm/yr)	13.1 ± 2.1	11.4 ± 2.5	10.8 ± 1.9	11.2 ± 2.3	0.016[b]	0.007
First year growth velocity SDS	5.1 ± 3.0	3.3 ± 2.9	3.2 ± 2.9	3.2 ± 2.9	ns	0.037

ns, Not significant.

[a] Tukey test: AA *vs.* AC, $P = 0.008$; AC *vs.* CC, $P = 0.63$; AA *vs.* CC, $P = 0.004$.

[b] Tukey test: AA *vs.* AC, $P = 0.05$; AC *vs.* CC, $P = 0.58$; AA *vs.* CC, $P = 0.015$.

TABLE 2. Clinical and auxological characteristics of 36 children with severe GHD who reached adult height after long-term treatment with rhGH, grouped according to −202 A/C *IGFBP3* genotype

	−202 A/C *IGFBP3* genotype			
	AA	**AC**	**CC**	**AC + CC**
n	9	19	8	27
Gender (males:females)	7:2	14:5	5:3	19:8
IGHD:CPHD	2:7	9:10	3:5	12:15
Target height SDS	−1.1 ± 0.9	−0.9 ± 0.7	−0.9 ± 0.8	−0.9 ± 0.7
Highest GH peak (μg/liter)	0.6 ± 0.7	1.1 ± 0.9	1.1 ± 1.0	1.1 ± 0.9
Chronological age (yr)	10.7 ± 4.2	10.5 ± 3.9	9.2 ± 2.4	10.1 ± 3.5
Bone age delay (yr)	5.8 ± 3.0	5.4 ± 3.0	4.1 ± 2.0	5.0 ± 2.9
Basal height SDS	−4.3 ± 1.1	−5.0 ± 1.8	−3.8 ± 1.1	−4.6 ± 1.7
Spontaneous:induced puberty	3:6	11:8	4:4	15:12
Age at start puberty (yr)	14.9 ± 2.9	15.2 ± 3.4	14.9 ± 3.7	15.1 ± 3.4
Treatment duration (yr)	8.3 ± 2.2	8.4 ± 3.3	9.9 ± 3.5	8.8 ± 3.3
Mean rhGH dose (μg/kg · d)	38 ± 5	36 ± 8	32 ± 10	35 ± 9
Final height SDS	−0.6 ± 0.6	−1.1 ± 1.1	−1.2 ± 1.6	−1.1 ± 1.2
Final height SDS−target height	0.5 ± 0.7	−0.1 ± 1.1	−0.4 ± 2.2	−0.2 ± 1.5

For all variables, comparison of genotypes (AA *vs.* AC *vs.* CC and AA *vs.* AC + CC) were not significant.

ment in IGFBP-3 SDS levels after treatment than patients presenting at least one C allele (Δ IGFBP-3 SDS, AA = 2.8 ± 1.4 *vs.* AC or CC = 1.6 ± 1.3; $P = 0.02$).

The observed relationship between IGFBP-3 serum levels during rhGH treatment and −202 A/C *IGFBP3* genotype was independent of many other clinical variables, such as age, sex, BMI, and rhGH doses, as demonstrated by multiple regression analyses. Alone, *IGFBP3* polymorphism accounted for 19% of IGFBP-3 serum level variation ($P < 0.001$) and, together with age ($P < 0.001$) and gender ($P = 0.003$), explained 54% of observed variation. Data analysis excluding IGF-I and IGFBP-3 measured by CLIA (about 10% of measurements) presented similar results (data not shown). There was no relation between −202 A/C *IGFBP3* genotype and IGF-I levels before the start of treatment, as well as during treatment (Table 1).

First year growth velocity

Mean growth velocity in the first year of rhGH treatment was higher in patients with the AA genotype and declined significantly in a stepwise manner in the presence of one or two copies of the C allele (first year growth velocity, AA = 13.0 ± 2.1 cm/yr, AC = 11.4 ± 2.5 cm/yr, and CC = 10.8 ± 1.9 cm/yr; $P = 0.016$) (Table 1 and Fig. 2). As a group, patients with at least one C allele presented a mean growth velocity 1.9 cm/yr lower than that of patients homozygous for the A allele (95% confidence interval for difference of means, 0.5 to 3.2 cm/yr; $P = 0.007$). Similar results were observed when growth velocities were analyzed as SDS adjusted for sex and age (Table 1).

Additionally, single followed by multiple linear regression analyses demonstrated that the influence of this polymorphism on growth velocity was independent of other variables. Alone,

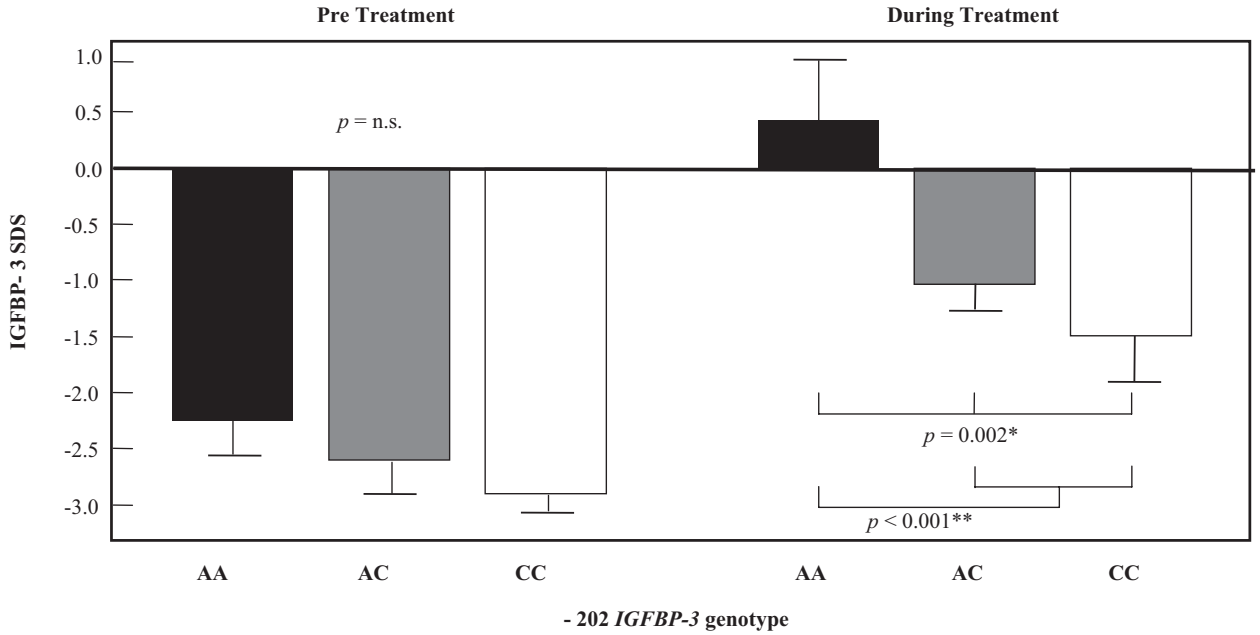

Fig. 1. Influence of the −202 A/C *IGFBP3* genotype on IGFBP-3 SDS levels in 71 prepubertal children with severe GHD before treatment and during rhGH treatment (mean ± SEM). *, AA *vs.* AC *vs.* CC; **, AA *vs.* AC + CC.

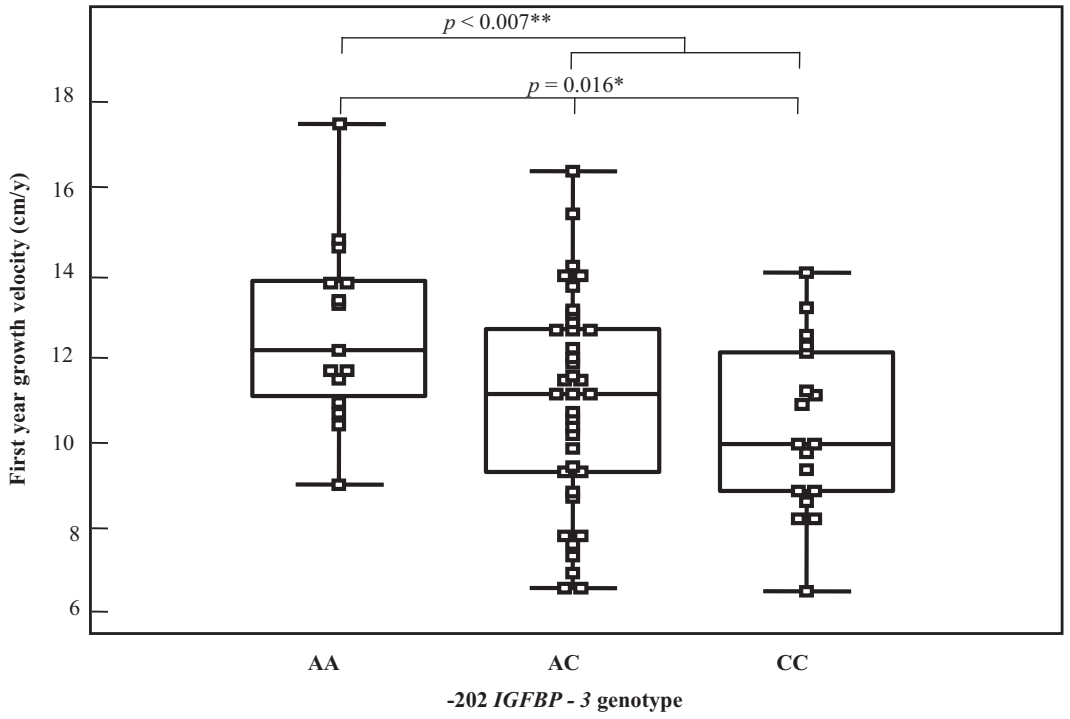

Fig. 2. Individual growth velocities of 71 prepubertal children with GHD during the first year of rhGH treatment, according to −202 A/C *IGFBP3* genotype. *, AA *vs.* AC *vs.* CC; **, AA *vs.* AC + CC.

IGFBP3 polymorphism accounted for 10% of growth velocity variation ($P = 0.004$) and together with height SDS ($P < 0.001$) and age at the start of rhGH therapy ($P = 0.001$), explained 29% of observed variation.

Final height SDS

Clinical and laboratory characteristics, as well as genotype distribution, of the 36 patients who reached adult height were indistinguishable from the 35 patients who were still on treatment. Genotypic groups were similar concerning gender distribution, isolated or combined GHD, spontaneous or induced puberty, age at puberty onset, parental height, highest GH peak, chronological age and bone age delay at the start of treatment, basal height SDS, BMI SDS, and mean rhGH doses (Table 2). Despite similar mean rhGH doses and treatment duration, there was a tendency for better final height growth outcomes in patients with the AA −202 *IGFBP3* genotype, but this difference was not significant (Table 2 and Fig. 3).

Discussion

Interest in a search for genetic variations that modulate rhGH sensitivity and help to explain the great variability in clinical outcomes observed in GHD children during rhGH therapy has increased in the last 5 yr. Genes involved in GH and IGF-I action are obvious candidates for rhGH pharmacogenetic studies. Considering that IGFBP-3 modulates both endocrine and local actions of IGF-I, and given the established functional importance of −202 A/C *IGFBP3* promoter region polymorphism, we evaluated whether this common polymorphism could influence growth responsiveness to rhGH therapy. Since 2001, genotype at this locus has been consistently correlated to circulating levels of IGFBP-3 in healthy adults (16). In the present study, the effects of this polymorphism on IGFBP-3 levels and growth response to rhGH treatment in children with GHD were assessed for the first time.

Regarding IGFBP-3 serum levels, studies in healthy adults demonstrated that individuals with the AA genotype present mean IGFBP-3 levels higher than patients with AC or CC genotypes (16–23). In our cohort of patients with severe GHD, this association became statistically significant only during rhGH treatment, which suggests that the effect of the −202 A/C *IGFBP3* polymorphism on IGFBP-3 levels may be at least in part dependent on GH action. Theoretically, IGFBP-3 serum levels are determined not only by gene transcriptional activity, but also by posttranslational changes and stabilization in ternary complexes. Other possible influential variables such as acid labile subunit and IGF-2 levels were not evaluated in the present study. However, functional studies and the consistency of correlations between −202 A/C *IGFBP3* genotype and IGFBP-3 serum levels in several studies (16–23) support a direct relationship between genotype at this locus and peptide levels.

In contrast with the clear influence of the −202 A/C genotype on IGFBP-3 serum levels, there was no correlation between this polymorphism and IGF-I serum levels, which is in accordance with other studies (20, 22) and could be explained by additional influential factors on IGF-I levels (27, 28).

Moreover, GHD children who harbor the −202 AA *IGFBP3* genotype presented better mean growth velocity during the first year of rhGH treatment than patients harboring −202 AC or CC *IGFBP3* genotype. The observed influence of genotype on

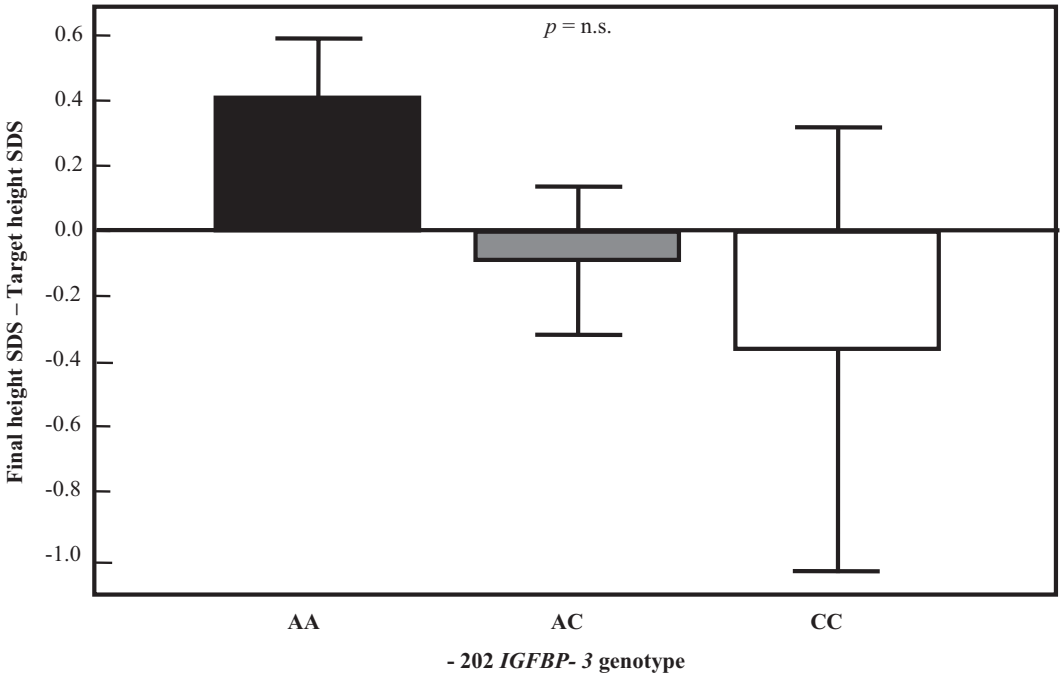

Fig. 3. Mean adult height SDS adjusted for target height SDS in 36 patients with severe GHD after long-term treatment with rhGH, according to −202 A/C *IGFBP3* genotype (mean ± SEM).

growth velocity was independent of other clinical variables, as demonstrated by multiple regression analyses including factors such as gender, target height SDS, age and height SDS at the start of treatment, GH peak at stimulation tests, and mean rhGH dose during treatment. Pretreatment growth velocity was not available for those analyses due to the severity of short stature in the majority of patients and the prompt GHD diagnosis.

A tendency for better adult height adjusted for target height in patients homozygous for the A allele was also observed, but this difference did not reach statistical significance. One previous study, especially designed to evaluate the association between *IGFBP3* polymorphisms and body size, found a weak association between the −202 A *IGFBP3* allele and higher adult height in a cohort of Hispanic, but not in non-Hispanic, women (29). A possible explanation for the lack of a clear association between this polymorphism and the height of healthy adults might be the fact that, in individuals with normal GH secretion, slight differences in GH sensitivity could be counterbalanced by a corresponding increase in GH secretion. Conversely, in patients with severe GHD, who depend on fixed doses of rhGH administered, the adaptive response is impaired, and differences in GH sensitivity will probably be more clearly observed. It is noteworthy that all our patients had severe GHD due to the stringent diagnostic criteria used in our unit. Hence, they comprise a homogenous severe GHD cohort, ideal to evaluate the effect of genetic factors on growth responsiveness to rhGH.

There is much evidence for stimulatory effects of IGFBP-3 on IGF-I action. First, IGFBP-3 prolongs the half-life of IGF-I, thus modulating its endocrine actions (11, 13). Moreover, IGFBP-3 can potentiate IGF-I autocrine and paracrine actions, as demonstrated by many *in vitro* (30–37) and *in vivo* studies (38, 39). Indeed, IGF-I complexed to IGFBP-3 appears to be more potent than free IGF-I under many conditions (36, 38–40). Some of the proposed mechanisms for stimulatory effects of IGFBP-3 on IGF-I actions are: 1) IGFBP interaction with cell or matrix components, which may concentrate IGFs near their receptor, enhancing IGF activity and facilitating the storage of IGFs in extracellular matrices for future action (11, 14); 2) accumulation of cell-bound forms of IGFBP-3 with lowered affinity for IGF, which may enhance the presentation of IGF to Type 1 IGF-receptor and facilitate a slow exchange of IGF-I between the receptor and IGFBP-3 (35, 41); 3) cell protection from IGF-I-mediated down-regulation of IGF-I receptor (33, 42); 4) potentiation of IGF action mediated through the phosphatidylinositol 3-kinase pathway (32).

In summary, we suggest that the higher levels of IGFBP-3, related to the presence of the −202 AA *IGFBP3* genotype, may result in prolonged half-life of the IGF-I/IGFBP-3 complex and/or amplification of IGF-I local effects, thus enhancing rhGH actions. For the first time, a direct relation between the −202 A/C *IGFBP3* promoter region polymorphism and responsiveness to rhGH treatment in GHD children was demonstrated. Patients homozygous for the A allele presented better growth velocities and higher IGFBP-3 levels in the first year of rhGH treatment than patients who harbor one or two copies of the C allele. These results substantiate the importance of pharmacogenetic studies on rhGH treatment and suggest that future studies adjusting rhGH treatment to genotype may provide additional tools to individualization of rhGH therapy and improved growth outcomes.

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