Pituitary diseases: long-term clinical consequences

Klaauw, A.A. van der

Chapter 14

Influence of the d3-growth hormone receptor isoform on short-term and long-term treatment response to growth hormone replacement in growth hormone deficient adults

Agatha van der Klaauw, Tahar van der Straaten, Renee Baak-Pablo, Nienke Biermasz, Henk-Jan Guchelaar, Alberto Pereira, Johannes Smit, Johannes Romijn

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ABSTRACT

Objective

Recombinant human growth hormone (rhGH) replacement in adults is aimed at improving signs and symptoms of the adult GH deficiency (GHD) syndrome. In children, a common polymorphism of the GH receptor (exon-3 deletion, d3GHR) increases the response to rhGH replacement. The aim of this study was to assess the effects of this polymorphism on the response to rhGH replacement in adults.

Design

Prospective intervention with rhGH during 1 year (n=99) and in a subset during 5 years (n=53).

Patients and methods

The presence of the d3GHR variant was established in GHD patients and linked to short-term and long-term effects of rhGH replacement on IGF-I, lipid metabolism, anthropometric parameters, and bone mineral density.

Results

Fifty-five patients had two wildtype alleles (56%), whereas 38 patients (38%) had one allele and 6 patients (6%) had two alleles coding the d3GHR isoform. During short-term rhGH replacement, the increase in IGF-I was higher in patients bearing at least one d3GHR allele compared to those with two wildtype alleles (at an identical mean dose of rhGH). The decrease in total cholesterol and LDL cholesterol was lower in the group bearing at least one d3GHR allele, whereas the increase in HDL cholesterol was higher compared to patients with the wildtype genotype. In contrast, these differential responses of GHR genotype could not be demonstrated during long-term rhGH replacement.

Conclusion

The d3GHR genotype contributes, at least for some parameters, to the interindividual differences in efficacy of short-term, but not of long-term, rhGH replacement in adults with GHD.

INTRODUCTION

The aim of recombinant human growth hormone (rhGH) replacement in adults is to ameliorate symptoms and signs of the adult growth hormone deficiency (GHD) syndrome. In adults with GHD these effects include beneficial effects in lipid concentrations, body composition, and bone mineral density (1-5).

Recently, a polymorphism in the growth hormone receptor, a genomic deletion of exon 3 (d3GHR), has been described to increase growth velocity during rhGH replacement in children with GHD (6) and idiopathic short stature or children who were short for gestational age (7). Due to this polymorphism GH signal transduction is enhanced despite unaltered GH receptor binding (8). The allele-prevalence is estimated to be 25-32% with a frequency of homozygosity of 9-14% (8;9). Consequently, this polymorphism might also contribute to inter-individual variability of the clinical response to rhGH replacement in adults with GHD.

Therefore, the aim of this study was to assess the effects of this common polymorphism on the response to short-term replacement (1 year) and, in a subset of patients, long-term rhGH replacement (5 years) with rhGH on IGF-I levels, anthropometric and metabolic parameters, and bone mineral density.

PATIENTS AND METHODS

Patients

All patients with GHD, visiting our outpatient clinic and in whom efficacy parameters of rhGH replacement were collected prospectively in a standardized manner, could be included in the present study (n=145). The exclusion criteria were known genetic defects in GH-IGF-I pathways (for example known mutations in the GHR).

All eligible patients were sent an extensive letter regarding the present protocol. Subsequently, the patients were contacted by telephone to ask consent for participation. Eleven patients declined participation, and 23 patients did not respond at all to repeated telephone calls or did not have updated telephone number. Consequently, saliva collection kits were sent to the home of the 111 remaining patients in prepaid envelopes. Ten of these 111 samples were not returned, and 2 saliva samples were not collected in a proper manner. Thus, genotyping could be performed in 99 patients. No differences were found in clinical characteristics between patients of whom DNA samples were received (n=101) and the remaining patients (n=44). Short-term effects could be evaluated in all 99 patients (Table 1), whereas in 53 of these 99 patients long-term effects could be evaluated due to an earlier starting date of rhGH treatment (Table 2).

Study design and treatment protocol

Patients were prospectively enrolled in an open label treatment protocol. Growth hormone deficiency (GHD) was confirmed in all patients by an insulin tolerance test (nadir blood glucose <2.2 mmol/l) with a peak GH concentration <3 µg/l. After initial measurements were obtained, all patients were treated with subcutaneous injections of rhGH (Genotropin® Pharmacia/Pfizer or Zomacton® Ferring, Norditropin® NovoNordisk, or Humatrope® Lilly), administered subcutaneously every evening. The initial dose of rhGH was 0.2 mg/day, which was individually adjusted each month in the first half year to achieve physiological serum IGF-I concentrations, within the age-dependent laboratory reference range (IGF-I standard deviation scores (SD scores)). Thereafter, this individualized dose was continued in each patient and adjusted, if necessary, to maintain a normal IGF-I concentration for the duration of the study.

Patients with a functioning adenoma (short-term group: 18 patients with Cushing's disease, 4 patients with acromegaly and 12 patients with prolactinoma and long-term subset: 7 patients with Cushing's disease, 2 patients with acromegaly and 7 patients with prolactinoma) were in remission before entering the study. The diagnosis of GHD after acromegaly was suspected in patients treated with postoperative radiotherapy and now suffering from panhypopituitarism and confirmed with an insulin-tolerance test (10). When secondary amenorrhoea was present for more than 1 year premenopausal women were defined as LH/FSH deficient. In men, LH/FSH deficiency was defined, as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a total or free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 µmol/l) after a corticotrophin releasing hormone stimulation test or insulin tolerance test. Conventional substitution therapy was monitored during substitution with rhGH and the respective dosages were adjusted, as required for normalization of clinical and biochemical parameters of pituitary deficiencies.

The study protocol was approved by the local Ethics Committee. All patients gave written informed consent to participation in the study.

DNA collection and genetic analysis

All patients, who consented on participation, received a saliva collection kit (Oragene kit Westburg, Leusden, The Netherlands). Participants were requested to provide 2 ml of saliva. DNA extraction was done 4 to 6 weeks after saliva collection and storage at room temperature. DNA concentrations and purity (OD260/280) were determined spectrophotometrically using the nanodrop (Isogen, IJsselstein, The Netherlands). The exon 3 deletion in the GHR gene was detected as described previously (10). Briefly, 25 ng DNA was PCR amplified using hotstart PCR mastermix (Qiagen, Venlo, The Netherlands) and primers G1, G2 and G3 (genbank asseccionnumber AF155912) as follows: initial denaturation of 15 minutes at 94°C, 35 cycles of 30s at 95°C, 30 s at 60°C and 2min at 72°C, followed by a final extension of 10min at 72°C. PCR

products were separated and visualized on an ethidiumbromide stained agarose gel. Expected allele frequencies were calculated by Hardy-Weinberg equilibrium.

Study parameters

Based on the genotype patients could be divided into two groups (group 1: homozygote wild type: two wildtype alleles, group 2: heterozygote (one d3GHR isoform, and one wildtype allele) and homozygote (two d3GHR isoforms).

Genotype was linked to:

1. Insulin-like growth factor-I (IGF-I) concentrations
2. Biochemical metabolic parameters: fasting levels of glucose, total cholesterol, HDL cholesterol (HDL), and triglycerides (TG). LDL cholesterol concentrations (LDL) were calculated using the Friedewald formula. Patients were requested to fast overnight before blood samples were taken for laboratory measurements of lipid profiles and glucose.
3. Anthropometric parameters: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressure (SBP and DBP respectively.) were measured. Body-mass index (BMI) and waist-hip (WH) ratio were calculated. Body weight was measured to the nearest 0.1 kg, and body height was measured barefoot to the nearest 0.001 m. The BMI was calculated as weight in kilograms divided by the square of height in meters.
4. Bone mineral density (BMD): BMD of the lumbar spine (L1-L4) was measured using dual-photon X-ray absorptiometry (DXA: Hologic QDR 1000 and 4500, Waltham, MA). During follow-up, the Hologic QDR 1000 was replaced by the Hologic QDR 4500. To be able to properly compare BMD scores, a conversion formula was developed based on data from an in–house comparison of both methods in 300 subjects (BMD LWK: Hologic 4500 (g/cm^2) =(0.9736*BMD Hologic QDR 1000)+0.0109).

Assays

Serum IGF-I (nmol/l) concentration was measured by RIA (INCSTAR Corp., Stillwater, MN) after extraction and purification on ODS-silica columns. The detection limit of this assay is 1.5 nmol/l, and the inter-assay coefficient of variation was below 11%. Age-adjusted IGF-I data were determined in the same laboratory (11;12). IGF-I concentrations were also expressed as SD scores for age-related normal levels.

A Hitachi 747 autoanalyzer (Roche, Mannheim, Germany) was used to quantify serum concentrations of glucose, total cholesterol and TG. HDL was measured with a homogenous enzymatic assay (Hitachi 911, Roche, Mannheim, Germany). In 2003 the Hitachi 747 was replaced by a modular P 800 with no change in the chemistry components.

Statistics

Statistical analysis was performed using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, Illinois, USA). Results are scored as the mean ± standard error of the mean, unless specified otherwise. Paired samples Student's t-test were used to compare baseline and 1 year (short-term) and baseline and 5 year rhGH replacement (long-term).

Patients were divided into two groups: 1) patients with two wildtype alleles and 2) patients carrying one allele coding for the d3GHR isoform and patients with two alleles coding for the d3GHR isoform combined. Differences for short-term and long-term rhGH replacement were calculated for all study parameters. Independent samples T-test were used to compare both groups. A p-value of <0.05 was assumed to represent a significant difference.

RESULTS

Patients

Short-term effects (1 year, Table 1): Ninety-nine patients (43 men) with a mean age of 51 years (range: 19-81 years) were studied. Fifty-five patients had two wildtype alleles (56%), whereas 38 patients (38%) had one allele coding the d3GHR isoform, and 6 patients (6%) had two alleles coding the d3GHR isoform. There were no differences in clinical characteristics between the two patient groups. Four patients were previously treated for acromegaly (n=2 wildtype, n=2 heterozygotes) and 18 patients were previously treated for Cushing's disease (n=10 wildtype, n=7 heterozygotes and n=1 homozygote).

At baseline, 9 patients with two wildtype alleles and 12 patients bearing at least one d3GHR allele used antihypertensive drugs (p=0.220), compared to 11 patients with two wildtype alleles and 14 patients bearing at least one d3GHR allele at follow-up (p=0.196). At baseline, 6 patients with two wildtype alleles and 5 patients bearing at least one d3GHR allele used lipid-lowering drugs (p=0.943), compared to 7 patients with two wildtype alleles and 7 patients bearing at least one d3GHR allele at follow-up (p=0.652).

Long-term effects (5 years, Table 1): Fifty-three patients (23 men) with a mean age of 49.4 years (range 19-70 years) were included in the long-term rhGH replacement pharmacogenetic analysis. Thirty-one patients had two wildtype alleles (58%), whereas 17 patients (32%) had one allele coding the d3GHR isoform, and 5 patients (9%) had two alleles coding the d3GHR isoform. There were no differences in clinical characteristics between the two patient groups. Two patients were previously treated for acromegaly (n=1 wildtype and n=1 heterozygote) and 7 patients were previously treated for Cushing's disease (n=4 wildtype, n=2 heterozygote and n=1 homozygote). At baseline, 4 patients with two wildtype alleles and 4 patients bearing at least one d3GHR allele used antihypertensive drugs (p=0.670), compared to 6 patients with two wildtype alleles and 7 patients bearing at least one d3GHR allele at follow-up (p=0.331). At baseline, 3 patients with two wildtype alleles and 2 patients bearing at least one d3GHR allele

Table 14/1: Clinical characteristics replacement in adults with growth hormone deficiency who completed short-term(n=99) and long-term (n=53) recombinant human growth hormone replacement: comparison between patients with two wildtype alleles (WT-WT), patients bearing one allele coding for the d3GHR isoform (d3GHR-WT), and patients bearing two d3GHR alleles (d3GHR-d3GHR).

		WT-WT (n=55)	d3GHR-WT and d3GHR-d3GHR (n=44)	P-value	WT-WT (n=31)	d3GHR-WT and d3GHR-d3GHR (n=22)	P-value
Gender (%)	Male/ Female	46/54	41/59	NS	45/ 55	41/59	NS
Age (years (mean ± SEM))		49.6 ± 1.5	52.7 ± 1.9	NS	50.6 ± 2.2	47.8 ± 2.9	NS
Etiology of GHD (%)	Non-functioning pituitary adenoma	29	36	NS	35	27	NS
	Functioning pituitary adenoma*	33	36		26	36	
	Craniopharyngioma	11	14		7	23	
	Other**	27	14		32	14	
Onset of disease (%)	Adult onset/ Childhood onset	96/4	95/5	NS	94/ 7	91/9	NS
Surgery (%)		78	80	NS	81	82	NS
Radiotherapy (%)		44	36	NS	42	41	NS
ADH deficiency (%)		31	23	NS	26	32	NS
ACTH deficiency (%)		78	75	NS	84	82	NS
TSH deficiency (%)		89	71	0.019	97	86	NS
LH/ FSH substitution therapy(%)	Men	80	67	NS	93	78	NS
	Women	53	42	NS	59	54	NS

*Prolactinoma, Cushing's disease, acromegaly.

**Cerebral malignancies and their treatment, traumatic brain injury, sheehan's syndrome, hypophysitis, midline defect, empty sella.

NS: non significant.

used lipid-lowering drugs (p=0.943), compared to 3 patients with two wildtype alleles and 4 patients bearing at least one d3GHR allele at follow-up (p=0.368).

Effects of rhGH replacement

Short-term effects

IGF-I concentrations significantly increased during 1 year of rhGH replacement. Glucose and HDL cholesterol concentrations increased during 1 year rhGH replacement, whereas total and LDL cholesterol concentrations decreased (Table 2). Triglyceride concentrations tended to increase during 1 year rhGH replacement. There were no changes in anthropometric parameters (SBP, DBP, waist circumference, WH ratio, BMI) or BMD.

To study variables that independently influenced short-term treatment effects, linear regression analysis with treatment endpoints which were influenced by short-term rhGH replacement (glucose, total cholesterol, LDL and HDL cholesterol) as dependent variables and the baseline value of the specific treatment parameter, age, gender (female (=0) vs. male (=1)), BMI at baseline, rhGH replacement dose, and diagnosis (no pituitary tumor (=0) vs. pituitary tumor (=1)) was performed. Glucose concentrations after 1 year rhGH replacement were affected by baseline glucose concentration (β=0.454, p<0.001) and baseline BMI (β=0.055, p=0.010). Total cholesterol after 1 year rhGH replacement was affected by baseline cholesterol (β=0.767, p<0.001), gender (β=-0.443, p=0.014) and diagnosis (β=0.479, p=0.019). HDL cholesterol after 1 year rhGH replacement was affected by baseline HDL (β=0.856, p<0.001), gender (β=-0.119, p=0.020), rhGH dose (β=0.425, p=0.007) and diagnosis (β=0.149, p=0.009). LDL cholesterol after 1 year rhGH replacement was only affected by LDL at baseline (β=0.741,p<0.001).

Long-term effects

IGF-I concentrations were significantly increased during 5 years rhGH replacement. Glucose levels tended to increase, although this difference did not reach statistical significance. Total and LDL cholesterol levels decreased significantly, whereas HDL increased despite a significant increase in BMI during long-term follow-up. Waist circumference and WH ratio, however, remained unchanged. In addition, DBP decreased, whereas SBP remained unaffected. BMD increased during 5 years of rhGH replacement. To study variables that independently influenced long-term treatment effects, linear regression analysis with treatment endpoints which were influenced by long-term rhGH replacement (total cholesterol, LDL and HDL cholesterol, DBP and BMD) as dependent variables and the baseline value of the specific treatment parameter, age, gender (female (=0) vs. male (=1)), BMI at baseline, rhGH replacement dose, and diagnosis (no pituitary tumor (=0) vs. pituitary tumor (=1)) was performed. None of these independent parameters apart from the individual baseline values of the parameters influenced the long-term endpoints (data not shown).

Table 14/2: Effects of recombinant human growth hormone (rhGH) replacement during short-term (1 year) and long-term (5 years) treatment in adults with growth hormone deficiency.

	Baseline	1 yr rhGH replacement	P-value*
Short-term (n=99)			
IGF-I (nmol/l)	10.1 ± 0.6	23.3 ± 1.0	<0.001
IGF-I SD score	-1.6 ± 0.1	1.0 ± 0.2	<0.001
Glucose (mmol/l)	4.7 ± 0.09	4.9 ± 0.09	0.007
Total cholesterol (mmol/l)	6.4 ± 0.1	6.1 ± 0.1	<0.001
LDL cholesterol (mmol/l)	4.7 ± 0.1	4.2 ± 0.1	<0.001
HDL cholesterol (mmol/l)	1.4 ± 0.05	1.5 ± 0.05	0.009
Triglycerides (mmol/l)	1.8 ± 0.1	1.9 ± 0.1	0.056
SBP (mm Hg)	133.8 ± 1.6	136.5 ± 2.1	NS
DBP (mm Hg)	84.0 ± 0.9	85.2 ± 1.0	NS
Waist circumference (cm)	95.3 ± 1.4	94.6 ± 1.4	NS
WH ratio	0.9 ± 0.009	0.9 ± 0.009	NS
BMI (kg/m^2)	27.1 ± 0.5	27.4 ± 0.5	NS
BMD (g/cm^2)	1.0 ± 0.1	1.0 ± 0.2	NS
Long-term (n=53)	Baseline	5 yr rhGH replacement	
IGF-I (nmol/l)	9.3 ± 0.7	22.6 ± 1.5	<0.001
IGF-I SD score	-1.8 ± 0.1	0.9 ± 0.3	<0.001
Glucose (mmol/l)	4.6 ± 0.1	4.9 ± 0.1	0.073
Total cholesterol (mmol/l)	6.8 ± 0.2	5.9 ± 0.2	<0.001
LDL cholesterol (mmol/l)	5.1 ± 0.2	4.0 ± 0.2	<0.001
HDL cholesterol (mmol/l)	1.4 ± 0.1	1.6 ± 0.1	<0.001
Triglycerides (mmol/l)	1.8 ± 0.1	2.0 ± 0.2	NS
SBP (mm Hg)	132.3 ± 2.0	129.7 ± 2.2	NS
DBP (mm Hg)	84.3 ± 1.2	81.5 ± 1.4	0.029
Waist circumference (cm)	92.7 ± 2.0	93.6 ± 2.1	NS
WH ratio	0.9 ± 0.01	0.9 ± 0.01	NS
BMI (kg/m^2)	26.0 ± 0.6	27.0 ± 0.7	0.006
BMD (g/cm^2)	0.9 ± 0.02	1.0 ± 0.03	0.011

Data are presented as mean ± SEM. *Baseline and 1 yr as well as baseline and 5 yr recombinant human growth hormone (rhGH) replacement data were compared with a paired samples T-test.
NS: non significant.

Pharmacogenetics during short-term rhGH replacement

No differences were found in the baseline values of the different metabolic parameters between the two groups (glucose, total/ LDL/ HDL cholesterol, and triglycerides).

However, the decrease in total cholesterol during 1 year rhGH replacement was markedly reduced in patients bearing at least one allele coding the d3GHR isoform compared to patients with two wildtype alleles (-0.08 ± 0.1 mmol/l in the d3GHR group vs. -0.5 ± 0.1 mmol/l in patients

Table 14/3: Changes in IGF-I, metabolic parameters, anthropometric parameters, and BMD during 1 year recombinant growth hormone replacement (short-term) and during 5 years in a subset of patients (long-term): comparison between patients with two wildtype alleles (WT-WT) and patients bearing at least one allele coding for the d3GHR isoform (d3GHR-WT and d3GHR-d3GHR).

	Short term treatment			Long-term treatment		
	WT-WT (n=55)	d3GHR-WT and d3GHR-d3GHR (n=44)	P-value	WT-WT (n=31)	d3GHR-WT and d3GHR-d3GHR (n=22)	P-value
IGF-I (nmol/l)	10.9 ± 1.1	15.9 ± 1.7	0.012	12.7 ± 2.0	14.1 ± 2.0	NS
IGF-I SD score	2.1 ± 0.2	3.2 ± 0.3	0.010	2.6 ± 0.4	2.9 ± 0.4	NS
Dose rhGH 1 yr (mg/day)	0.4 ± 0.02	0.4 ± 0.03	NS	0.5 ± 0.04	0.5 ± 0.05	NS
Glucose (mmol/l)	0.3 ± 0.1	0.2 ± 0.1	NS	0.1 ± 0.1	0.4 ± 0.3	NS
Total cholesterol (mmol/l)	-0.5 ± 0.1	-0.08 ± 0.1	0.010	-0.9 ± 0.3	-0.4 ± 0.2	NS
LDL cholesterol (mmol/l)	-0.6 ± 0.1	-0.2 ± 0.1	0.028	-1.0 ± 0.2	-0.7 ± 0.3	NS
HDL cholesterol (mmol/l)	0.01 ± 0.03	0.1 ± 0.03	0.012	0.1 ± 0.05	0.2 ± 0.1	NS
Triglycerides (mmol/l)	0.2 ± 0.1	0.002 ± 0.1	NS	0.1 ± 0.2	0.3 ± 0.2	NS
SBP (mmHg)	0.9 ± 3.1	4.9 ± 2.8	NS	-4.8 ± 2.0	0.6 ± 3.3	NS
DBP (mmHg)	0.6 ± 1.2	1.9 ± 1.8	NS	-3.1 ± 1.5	-2.4 ± 2.1	NS
Waist circumference (cm)	-1.1 ± 0.7	-0.2 ± 0.8	NS	1.2 ± 1.2	0.5 ± 1.4	NS
WH ratio	-0.004 ± 0.008	-0.002 ± 0.009	NS	0.02 ± 0.01	-0.03 ± 0.02	0.030
BMI (kg/m^2)	0.2 ± 0.2	0.4 ± 0.3	NS	0.9 ± 0.5	1.2 ± 0.3	NS
BMD (g/cm^2)	0.0004 ± 0.007	0.005 ± 0.007	NS	0.05 ± 0.02	0.04 ± 0.01	NS

Data are presented as mean ± SEM. NS: non significant.

with two wildtype alleles, p=0.010, Table 3, Figure 1). The reduction in LDL cholesterol was also lower in patients bearing at least one allele coding the d3GHR isoform compared to patients with two wildtype alleles (-0.2 ± 0.1 mmol/l vs. -0.6 ± 0.1 mmol/l, p=0.028). HDL increased more in patients bearing one allele coding the d3GHR isoform compared to patients with two wild-type alleles (0.1 ± 0.03 mmol/l vs. 0.01 ± 0.03 mmol/l, p=0.012). An additional analysis excluding all patients who used lipid-lowering drugs during follow-up, did not alter the conclusions.

No differences were found in baseline parameters or change during 1 year rhGH replacement in serum triglycerides or the anthropometric parameters (SBP, DBP, waist circumference, WH ratio, and BMI) between the two groups.

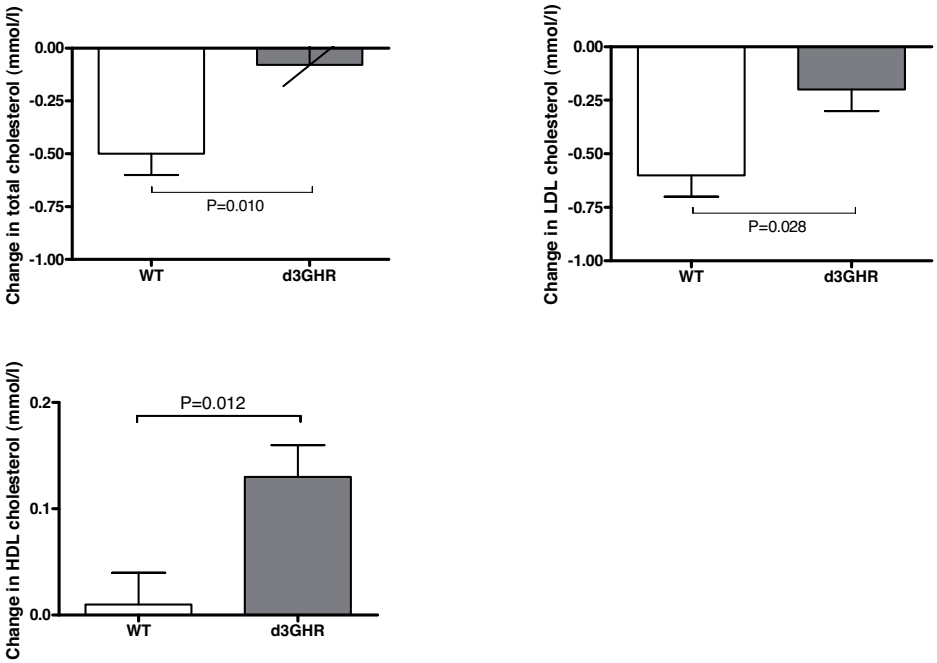

Figure 14/1: Change in IGF-I standard deviation score (SDS) total cholesterol, LDL cholesterol and HDL cholesterol levels during short-term rhGH replacement: comparison between patients with two wildtype alleles (WT-WT) and patients bearing at least one allele coding for the d3GHR isoform (d3GHR-WT and d3GHR-d3GHR).

No differences were found in BMD at the lumbar spine at baseline between the three groups, or during 1-year rhGH replacement.

Pharmacogenetics of long-term rhGH replacement

During long-term follow-up, no differences in treatment efficacy could be discerned between the two groups (Table 3). IGF-I concentrations and IGF-I SD scores were increased to a similar extent during long-term follow-up with no difference between rhGH replacement dose at 5 years. WH ratio decreased in patients bearing at least one allele of the d3GHR whereas it increased slightly in patients with two WT alleles, without any differences in waist circumference at baseline or follow-up or change in waist circumference. None of changes in the other biochemical metabolic parameters, anthropometrics measurements, and BMD differed between the two groups.

DISCUSSION

In this study, we evaluated the pharmacogenetics of rhGH replacement in adult patients with GHD according to d3GHR genotype. We found that the d3GHR genotype was associated with differences in efficacy during short-term, but not during long-term, rhGH replacement. These results suggest that the d3GHR genotype could contribute, at least for some parameters, to the inter-individual differences seen in efficacy of rhGH replacement in adults with GHD.

Various studies have assessed the interaction between efficacy of rhGH replacement and d3GHR polymorphisms in various groups of GHD and non-GHD children (6;13-18). The results in GHD children are conflicting. One study found a higher growth velocity in the group bearing at least one d3-GHR allele (6), whereas two other studies did not show this relationship (15;18).

The efficacy parameters of rhGH replacement in children with GHD are clear cut: growth velocity and final height. In adults with GHD the effects of rhGH replacement include more subtle effects in lipid concentrations, body composition, and bone mineral density (1-5). In this study, we focused on biochemical and anthropometric data and bone mineral density during short-term (1 year) and long-term (5 years) rhGH replacement in GHD adults. The increase in IGF-I levels was remarkably higher during short-term rhGH replacement in heterozygous patients bearing at least one allele of the d3GHR compared to patients bearing two wildtype alleles despite the fact that patients were treated with exactly the same dose of rhGH. This enhanced IGF-I generation upon stimulation with rhGH is in line with the higher IGF-I increment during an IGF-I generation test in children with idiopathic short stature bearing the d3GHR allele (19). In addition, in patients with acromegaly, a lower GH concentration was required in carriers of the d3GHR allele to produce a given increase in serum IGF-I concentrations (20). After long-term rhGH replacement, however, we did not observe such a pharmacogenetic effect of rhGH on IGF-1 levels. We speculate that downregulation of the GH-IGF-I system via negative feedback mechanisms might be involved to explain this discrepancy between short- and long term treatment with rhGH. Additionally, the fact that rhGH doses were individualized to achieve normal IGF-I levels could explain the lack of correlation between the GHR polymorphism and specific long-term endpoints.

In addition to these pharmacogenetic differences in IGF-I increase during short-term rhGH replacement between the two different genotypes, lipid parameters were differentially influenced by short-term rhGH replacement. The decrease in total cholesterol and LDL cholesterol during short-term rhGH replacement, was significantly lower in patients bearing at least one d3GHR allele compared to patients bearing two wildtype alleles. Moreover, the increase in HDL cholesterol during rhGH replacement, was significantly higher in patients bearing at least one d3GHR allele compared to patients bearing two wildtype alleles.

GH and IGF-I both have effects on lipid metabolism. In addition to stimulating lipolysis and thereby increasing plasma free fatty acid availability, GH increases the number and activity of hepatic LDL receptors, which enables LDL catabolism (21). In accordance, GH treatment in

mice with genetic LDL receptor defects does not lower plasma LDL concentrations (22). GH also increases the activity of cholesterol 7α-hydroxylase, the rate limiting enzyme in bile acid synthesis (23). These effects contribute to the decrease of total cholesterol and LDL cholesterol seen during short-term as well as long-term rhGH replacement. On the other hand, growth hormone enhances the expression of mRNA of sterol regulatory element-binding protein 1c (SREBP-1c), involved in hepatic lipogenesis (24). Furthermore, IGF-I suppresses scavenger receptor of class BI (SR-BI) (25). The SR-BI is expressed in liver and steroidogenic tissues and clears the HDL cholesterol from the circulation (25). These two latter effects of GH and IGF-I on lipogenesis and HDL expression in light of the enhanced signal transduction of the d3GHR variant and the increased IGF-I response in our patients, might thus contribute to the differential effects of the genotype on short-term rhGH replacement effects on lipid metabolism. In the long-term, these effects might be overruled by negative feedback signals due to changes in fat mass during rhGH replacement (2;26). Detailed studies on lipid metabolism in adult patients with the d3GHR genotype compared to patients with the wildtype genotype are warranted.

Some factors may have influenced our results. Only 6 patients were homozygous for the d3GHR variant, in accordance with the estimated prevalence in the general population (8;10). Therefore, we chose to evaluate those patients and patients heterozygous for the d3GHR variant together. Larger, probably multicenter studies are warranted to tease out the additional differences between patients heterozygous and homozygous for the d3GHR allele.

In conclusion, the d3GHR genotype is associated with differences in efficacy of short-term rhGH replacement with respect to IGF-I and lipid metabolism. These results suggest that the d3GHR genotype could contribute, at least for some parameters, to the inter-individual differences seen in efficacy of rhGH replacement in adults with GHD.

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