Impact of IGF(CA)19 gene polymorphism on the metabolic response to GH therapy in adult GH-deficient patients

C Giavoli^{1,2}, E Profka^{1,2}, E Sala^{1,2}, M Filopanti^{1,2}, A M Barbieri^{1,2}, S Bergamaschi^{1,2}, E Ferrante^{1,2}, M Arosio^{1,3}, B Ambrosi⁴, A G Lania^{5,6}, A Spada^{1,2} and P Beck-Peccoz^{1,2}

¹Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy, ²Endocrinology and Diabetology Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Via Francesco Sforza, 35, 20122 Milan, Italy, ³Unit of Endocrine Diseases and Diabetology, Ospedale San Giuseppe, Multimedica, Milan, Italy, ⁴Endocrinology and Diabetology Unit, Department of Medical and Surgical Sciences, University of Milan, IRCCS Policlinico San Donato, San Donato Milanese, Milan, Italy ⁵Biometra Department, University of Milan, and ⁶Endocrine Unit, Humanitas Clinical and Research Center, Rozzano, 20089 Milan, Italy

Correspondence should be addressed to C Giavoli
Email
claudiagiavoli@yahoo.it

Abstract

Objective: A polymorphism in the promoter region of the *IGF1* gene has been linked to serum IGF1 levels, risk of diabetes, and cardiovascular diseases with conflicting results. The aim of this study was to investigate the impact of this polymorphism on the short-term (1 year, $n=98$) and long-term (5 years, $n=50$) metabolic response to recombinant human GH (rhGH) in GH-deficient (GHD) adults.

Design and methods: Prospective study on GHD adults. Different genotypes were studied by microsatellite method. According to the most frequent 192 bp allele (19 cytosine–adenosine-repeats), subjects were divided into homozygous (19/19), heterozygous (19/X), and noncarriers (X/X).

Results: Basal characteristics of patients as well as their response to rhGH in terms of decrease in body fat percentage and increase in IGF1 levels were not different in the three genotype-groups. Conversely, after 1-year rhGH, a significant worsening of insulin sensitivity (i.e. increase in fasting glucose levels and homeostasis model assessment of insulin resistance) and a significant improvement in lipid profile (i.e. reduction in total cholesterol and LDL-cholesterol) were recorded only in homozygous subjects. In the long-term, insulin sensitivity was restored in all the patients, while a significant improvement in lipid profile was observed in homozygous and heterozygous subjects, but not in noncarrier subjects. No difference in rhGH dose among groups was recorded throughout the study.

Conclusions: In GHD adults, the presence of the WT allele in the IGF1 gene promoter may enhance sensitivity to either negative or positive metabolic changes induced by rhGH.

European Journal of
Endocrinology
(2014) 170, 273–281

Introduction

Growth hormone deficiency (GHD) in adults is a clinical syndrome characterized by several metabolic alterations such as increased body fat percentage (BF%), impaired physical performance, altered lipid profile, and insulin resistance. Many reports support efficacy of recombinant human GH (rhGH) replacement therapy in reversing most of the above-mentioned alterations (1, 2, 3). As suggested

by recent guidelines (4), GH replacement in adults should be started with low doses, thereafter titrated and individualized according to insulin-like growth factor 1 (IGF1) levels and clinical conditions of the patients (4, 5), in order to obtain the best efficacy, minimizing side-effects. Nowadays, it is well established that the individual response to rhGH is highly variable and, in the last years,

some pharmacogenetics studies have tried to find a possible explanation for this inter-individual variability, as genetic factors could play a role in the response to rhGH in GHD adults.

Namely, a common polymorphism of the GH receptor (GHR d3/fl) has been extensively studied. As the d3-GHR isoform has been shown to have an increased receptor activity due to an enhanced signal transduction (6, 7), various authors have studied the relationship between the efficacy of rhGH therapy and this polymorphism, especially in children, as summarized in a quite recent systematic review by Wassenaar *et al.* (8). Regarding adults, our group has recently reported that the functional difference of d3-GHR may confer major sensitivity to metabolic effects of rhGH in GHD adults (9).

As IGF1 is the main GH effector, a number of studies have also examined polymorphisms of the *IGF1* gene. In particular, a highly polymorphic microsatellite comprising a variable length of a cytosine-adenosine (CA) repeat sequence ($n=10-24$) has been identified in the promoter region of *IGF1* gene, 1 kb upstream from the transcription site. The number of CA repeats ranges between 10 and 24 and the most common allele in the Caucasian population contains 19(CA) (192 bp) repeats (10). This microsatellite has been linked to IGF1 levels, risk of diabetes, and cardiovascular diseases in different populations, with conflicting results (10, 11, 12, 13, 14, 15, 16).

Regarding pathological conditions of GH secretion, various studies have been performed in short children while in adults, there are up to now two reports in the literature about this topic. In particular, Akin *et al.* (17) evaluated a cohort of acromegalic subjects and found that patients having >194 bp genotype (more than 20 CA repeats) have higher IGF1 levels and require higher dose medication than patients with <192 or 192–194 bp genotype. In turn, Meyer *et al.* (18) investigated a possible association between the 19(CA) polymorphism and GH dose in GHD adults and did not find any correlation between the number of CA repeats and the responsiveness to rhGH, at least in terms of IGF1 levels. No data are so far available about an eventual relationship between this polymorphism and the metabolic changes induced by rhGH in GHD adults, changes known to differ from patient-to-patient. Thus, the aim of this study was to investigate the impact of the IGF(CA)19 gene polymorphism on basal phenotype and on short- and long-term response to rhGH in GHD adults, especially in terms of body composition, glucose, and lipid metabolism.

Subjects and methods

Patients and study design

This was a prospective study on 98 GHD adults ($M=60$, $F=38$, mean age 46 ± 13 years). Ninety-nine healthy age and sex-matched subjects were recruited as controls for genetic analysis. Diagnosis of severe GHD was defined by a GH peak $<3 \mu\text{g/l}$ to insulin tolerance test (19) or $<11.5 \mu\text{g/l}$ if BMI was below 25 kg/m^2 , $<8.0 \mu\text{g/l}$ if BMI was between 25 and 30 kg/m^2 , and $<4.2 \mu\text{g/l}$ if BMI was over 30 kg/m^2 to arginine+GHRH test (20). The causes of GHD were nonfunctioning pituitary adenoma ($n=33$), prolactinoma ($n=20$), craniopharyngioma ($n=16$), GH-secreting adenoma ($n=8$), Cushing's disease ($n=4$), idiopathic ($n=7$), primary empty-sella ($n=4$), traumatic brain injury ($n=2$), Rathke cleft cysts ($n=2$), and other hypothalamic-pituitary diseases ($n=2$, one meningioma and one histiocytosis). All patients with childhood-onset GHD ($n=10$) were appropriately retested before the beginning of rhGH replacement in adult life. Seven patients had isolated GHD and 91 had multiple pituitary hormone deficiencies variously associated. Diabetes insipidus was present in 22% of subjects. Pituitary function was determined with basal or dynamic evaluation, as appropriate. In particular, central hypothyroidism was defined as low free thyroxine (FT_4) levels in the presence of inappropriate thyroid-stimulating hormone levels. Central hypoadrenalism was defined as a lack of response to stimulation tests (peak cortisol levels lower than 500 nmol/l during standard-dose ($250 \mu\text{g}$) corticotropin stimulation test or insulin tolerance test). Central hypogonadism was diagnosed in case of low testosterone or estradiol levels and inappropriately normal or low gonadotropin levels. When necessary, conventional hormone replacement therapy for other pituitary hormone deficiencies was given at stable doses for at least 3 months before beginning rhGH therapy. In particular, all patients with central hypoadrenalism received a dose of 25 mg/day cortisone acetate, while other replacement therapies were adjusted according to hormonal results. Moreover, as it is known that rhGH therapy may unmask or worsen a central hypothyroid or hypoadrenal state (21, 22), both hypothalamus-pituitary-thyroid and -adrenal axis were re-evaluated not later than 6 months after the beginning of rhGH and replacement therapy was started or adjusted, when necessary. One-year effects were evaluated in all 98 patients, whereas in 50 of them a 5-year follow-up was available due to an earlier starting date of rhGH. Initial mean rhGH dose was 0.2 and 0.3 mg/day in men and in

women, respectively, then individually titrated against IGF1 levels between 0 s.d. and below the upper limit of the age- and sex-related reference range, as evaluated after 3 months of therapy. Mean rhGH dose was 0.31 ± 0.17 and 0.36 ± 0.17 mg/day after 1 and 5 years respectively. There was no difference in rhGH dose among the three genotype-groups at any time of the study. Informed consent was obtained from all participants and the study was approved by the Local Ethics Committee.

Study parameters and assays

In all the patients, several metabolic parameters such as serum fasting glucose (FG) and fasting insulin (FI), HbA1c, and lipid profile (total cholesterol and HDL-cholesterol, triglycerides (TG)) were evaluated. Insulin resistance degree was determined using the homeostasis model assessment of insulin resistance ($\text{HOMA-IR} = \text{FI (mU/l)} \times \text{FG (mmol/l)} / 22.5$) (23). Serum IGF1 levels were measured by a chemiluminescent immunometric assay (Immulite 2000 IGF1; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), with an intra- and interassay coefficient of variation of 2.9 and 7.4% respectively. IGF1 SDS was calculated from age- and sex-dependent reference ranges. All the other biochemical parameters were measured by standard procedures. LDL-cholesterol levels were evaluated by the formula: $\text{LDLc} = \text{total cholesterol} - \text{HDLc} - \text{TG}/5$ (24).

Anthropometric measurements

Body composition was evaluated by whole-body bioelectrical impedance analysis, using a portable impedance analyzer (RJL Systems, Detroit, MI, USA), following the instruction given by the manufacturer. BF% was calculated using Segal's regression equation (25) and the results were compared with those reported by Pichard *et al.* (26) in normal subjects matched for age and sex. BMI was calculated as weight in kilograms divided by the square of height in meters.

DNA extraction and genetic analysis

In all 98 GHD patients and in 99 control subjects, leukocyte DNA was extracted from blood samples using Nucleon BACC2 genomic DNA purification kit (GE Healthcare, Piscataway, NJ, USA) in compliance with the manufacturer's instructions. A multiplex PCR was carried out to determine the IGF(CA)19 gene polymorphism using a specific primer for the IGF1(CA)19 area (forward primer, 5'-FAM-GCT AGC

CAG CTG GTG TTA TT-3' and reverse primer, 5'-ACC ACT CTG GGA GAA GGG TA-3'). PCR was carried out in a 25 μ l reaction mix with Platinum Taq (Invitrogen) and subjected to denaturation at 96 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 59 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min. Amplification products were subsequently run on 1% agarose gel electrophoresis. One microliter of PCR product was then loaded into a 96 well-plate with the specific markers of known molecular weights to then proceed to the analysis of the size/height/area of the peaks generated by the electrophoretic run. In each well of the plate were added 1 μ l PCR product, 0.2 μ l Gene ScanLiz 500-SIZE STANDARD (Applied Biosystems), and 10 μ l formamide. The well-plate was then analyzed by capillary electrophoresis using a 3100 Genetic Analyzer (Perkin-Elmer Corp., Waltham, MA, USA). All raw data were analyzed with the bioinformatic software Peak Scanner, version 1.0 (Applied Biosystems).

Statistical analysis

Calculations were performed by SPSS for Windows, version 17.0 (SPSS). The data are expressed as mean $\pm$ s.d., whereas proportion and frequencies were used for categorical variables. Normal distributed variables were compared using Student's *t*-test among the groups of patients and within each group at 0–1–5 years. Levene's test was performed first to check the equality of variances. Categorical variables were compared by Fisher or χ^2 -test, where appropriate.

In order to assess the role of confounders, i.e. sex, age at diagnosis, and metabolic syndrome (MS), linear multiple regression analyses were carried out on metabolic parameters measured during follow-up. A two-tail $P < 0.05$ was considered statistically significant. In multiple comparisons tests, Bonferroni's correction of α value was used.

Results

Baseline: GHD genotype and phenotype

According to the most frequent 19(CA) repeat allele (192 bp), patients were divided into homozygous (19/19, $n = 38$, 38.7%), heterozygous (19/X, $n = 44$, 44.8%), and noncarriers (X/X, $n = 16$, 16.3%). Allele frequency was not different in GHD patients compared with controls and conformed with the Hardy–Weinberg equilibrium.

At baseline, most of the patients had IGF1 levels below the normal range for age and sex (61%), in particular IGF1

levels were low in 30 out of 38 (78%), 30 out of 44 (68%), and nine out of 16 (56%) patients of 19/19, 19/X, and X/X groups respectively. Though these proportions seemed to follow a decreasing trend from patients homozygous to patients noncarrying the WT allele, no significant difference was observed at χ^2 -test ($P=0.54$). BF% was significantly higher in noncarriers than in heterozygous and homozygous (37 ± 8 vs 30 ± 9 and 31 ± 9 respectively, $P<0.02$) subjects.

No differences were found in FG, HbA1c, insulin, total cholesterol, HDL-cholesterol, and (TG) levels among the three genotype-groups. Demographic and clinical data, as well as comparison of basal parameters among the three different genotypes, are presented in Table 1.

According to previously established criteria (27), diagnosis of MS was made in nine patients (one in 19/19, three in 19/X, and five in X/X). Prevalence of MS was significantly higher in the X/X group ($P<0.001$ at χ^2 -test). Basal prevalence of MS as well as of its individual components is presented in Table 1. At the beginning of the study, 12 subjects (three in 19/19, five in 19/X, and four in X/X group) were taking lipid-lowering agents and 12 (six in 19/19, three in 19/X, and three in X/X group) were taking antihypertensive drugs. These therapies remained unchanged throughout the study.

One-year period: effects of rhGH and pharmacogenetics

After 1 year of rhGH replacement, in the whole cohort of patients, mean IGF1 levels significantly increased (IGF1 SDS from -2.0 ± 0.9 to -0.4 ± 1.2 , $P<0.01$) and BF% significantly decreased (from 32 ± 8 to $30 \pm 8\%$, $P<0.01$). Regarding metabolic parameters, FG and FI significantly increased (from 81 ± 9 to 85 ± 9 mg/dl and from 9.3 ± 7 to 11.2 ± 9.3 μ U/ml respectively, $P<0.01$), thus reflecting an initial worsening of insulin sensitivity, as mirrored by a significant increase in HOMA-IR (from 1.9 ± 1.5 to 2.4 ± 1.9 , $P<0.01$). Total cholesterol and LDL-cholesterol significantly decreased (from 215 ± 44 to 201 ± 42 and from 136 ± 43 to 121 ± 40 mg/dl respectively, $P<0.01$), while HDL-cholesterol did not change. Interestingly, when analyzing rhGH effects according to the three IGF1 genotypes, while the magnitude and significance of increase in IGF1 levels were not different, the worsening of insulin sensitivity and the improvement in lipid profile were significantly different only in homozygous patients. Statistical analysis among the three genotype-groups did not reveal any significant differences in 1 year rhGH effects on the evaluated parameters. As observed at baseline in noncarriers, a higher BF% (28 ± 8 , 27 ± 9 , and $35 \pm 8\%$ in 19/19, 19/X, and X/X respectively, $P<0.01$) and a higher

Table 1 Clinical characteristics of the 98 GHD patients and basal prevalence of MS and of its individual components: comparison between the three genotype-groups.

	19/19 (n=38)	19/X (n=44)	X/X (n=16)
Clinical characteristics of the 98 GHD patients			
Age (years)	47 ± 15	45 ± 15	44 ± 13
Sex (M/F)	27/11	28/16	6/10
BMI (kg/m ²)	27 ± 4	26 ± 6	27 ± 5
BF%	31 ± 9	30 ± 9	$37 \pm 8^*$
IGF1 (SDS)	-2.0 ± 1.1	-1.9 ± 0.9	-1.9 ± 0.7
HbA1c (%)	5.0 ± 0.6	4.9 ± 1.1	4.9 ± 0.8
FG (mg/dl)	82 ± 8	78 ± 17	83 ± 10
FI (μ U/ml)	8 ± 5	9 ± 5	10 ± 5
HOMA	1.7 ± 1.0	1.8 ± 1.6	2.1 ± 1.1
T-chol (mg/dl)	215 ± 43	215 ± 44	216 ± 53
HDL-chol (mg/dl)	53 ± 17	51 ± 17	51 ± 14
TG (mg/dl)	131 ± 53	131 ± 66	147 ± 60
LDL-chol (mg/dl)	138 ± 38	137 ± 41	136 ± 52
Basal prevalence of MS and of its individual components			
HDL <40 (M) and <50 (F) (mg/dl)	37	36	19
TG >150 (mg/dl)	24	23	31
FG >110 (mg/dl)	0	0	0
WC >102 (M) >88 (F) (cm)	18	20	56
MS	3	7	31*

* $P<0.01$ X/X vs 19/X and 19/19. M, male; F, female; BF, body fat; FG, fasting glucose; FI, fasting insulin; T-chol, total cholesterol; TG, triglycerides; WC, waist circumference; BP, blood pressure; MS, metabolic syndrome.

MS prevalence than in other genotype groups ($n=10$, two in 19/19, one in 19/X, and seven in X/X, $P<0.001$ at χ^2 -test) were observed. Distribution and prevalence of the individual components of the MS did not change after the 1-year rhGH treatment period (data not shown).

Regarding hypothalamic–pituitary–adrenal and –thyroid axis, central hypoadrenalism was newly diagnosed in six patients (two in 19/19, three in 19/X, and one in X/X group), in whom hydrocortisone replacement was promptly started and central hypothyroidism, documented by low FT₄ levels, was detected in ten patients (four in 19/19, four in 19/X, and two in X/X) and L-T₄ therapy was started ($n=7$) or increased ($n=3$) as needed.

The present results still hold significance after consideration of possible confounders such as sex, age at diagnosis, and presence of MS and BF%. No difference in rhGH dose was observed among the three groups. GH effects in the three different genotypes are presented in Table 2.

Five-year period: effects of rhGH and pharmacogenetics

In the 50 patients evaluated after long-term treatment, IGF1 normalization was maintained and BF% further decreased (from 32 ± 8 to 29 ± 8 and to $27\pm 8\%$, at baseline and after 1 and 5 years respectively, $P<0.01$). Similarly, the reduction in total cholesterol and LDL-cholesterol observed at 1 year was confirmed after 5 years (from 208 ± 50 to 191 ± 41 mg/dl and from 135 ± 44 to 111 ± 6 mg/dl respectively, $P<0.01$). Analyzing separately the three genotype-groups, improvement in lipid profile

was significant only in 19/19 and 19/X patients, i.e. in those bearing at least a WT allele. As far as rhGH effects on glucose metabolism on the whole cohort, both FG and FI levels after a short-term increase returned toward baseline (FG, from 80 ± 8 to 86 ± 7 to 83 ± 8 mg/dl, at baseline and after 1 and 5 years, respectively, $P<0.01$ 1 year vs baseline, $P=NS$ 5 years vs baseline; FI, 10.9 ± 8 to 13.1 ± 9 to 10.1 ± 8 μ IU/ml at baseline and after 1 and 5 years, respectively, $P<0.01$ 1 year vs baseline, $P=NS$ 5 years vs baseline), thus reflecting a long-term restoration of insulin sensitivity. However, evaluating rhGH effects according to the genotype, in the 19/19 patients, FG levels remained significantly higher than at baseline, as observed in the short-term metabolic response. Contrary to what has been observed at baseline and after 1 year, BF% in the X/X group, though slightly higher, was not significantly different from 19/19 and 19/X groups, while MS prevalence was still higher in noncarriers than in other genotype groups ($n=9$, one in 19/19, two in 19/X, and nine in X/X, $P<0.001$ at χ^2 -test). Distribution and prevalence of the singular components of the MS did not change after the 5-year rhGH treatment period (data not shown). No difference from basal conditions was observed in any of the other evaluated parameters. No difference in rhGH dose was observed among the three groups. Statistical analysis between the three genotype-groups did not reveal any significant differences in 5 year-rhGH effects on the evaluated parameters.

The present results still hold significance after consideration of possible confounders such as sex, age at diagnosis, and presence of MS and BF%. GH effects in the different genotype-groups are presented in Table 3.

Table 2 Effects of rhGH during short-term (1 year) treatment in GHD adults ($n=98$) according to different *IGF1* gene promoter genotypes. Significant metabolic changes are shown in bold.

	19/19 ($n=38$)		19/X ($n=44$)		X/X ($n=16$)	
	Baseline	1 year	Baseline	1 year	Baseline	1 year
BMI (kg/m ²)	27 ± 4	27 ± 4	26 ± 6	26 ± 7	27 ± 5	28 ± 5
BF%	31 ± 9	$28\pm 8^{\dagger}$	30 ± 9	$27\pm 10^{\dagger}$	$37\pm 8^*$	$35\pm 8^{*,\dagger}$
IGF1 (SDS)	-2.0 ± 1.1	$-0.3\pm 1.2^{\dagger}$	-1.9 ± 0.9	$-0.5\pm 1.2^{\dagger}$	-1.9 ± 0.7	$-0.3\pm 1.2^{\dagger}$
HbA1c %	5.0 ± 0.6	5.1 ± 0.6	4.9 ± 1.1	4.9 ± 1.2	4.9 ± 0.8	4.9 ± 0.9
FG (mg/dl)	82 ± 8	$86\pm 9^{\dagger}$	78 ± 17	$80\pm 20^{\dagger}$	83 ± 10	86 ± 8
FI (μ IU/ml)	8 ± 5	$10\pm 6^{\dagger}$	9 ± 5	10 ± 9	10 ± 5	11 ± 5
HOMA	1.7 ± 1.0	$2.4\pm 1.7^{\dagger}$	1.8 ± 1.6	2.3 ± 1.9	2.1 ± 1.1	2.4 ± 1
T-chol (mg/dl)	215 ± 43	$193\pm 40^{\dagger}$	215 ± 44	207 ± 39	216 ± 53	206 ± 52
HDL-chol (mg/dl)	53 ± 17	56 ± 23	51 ± 17	52 ± 14	51 ± 14	51 ± 16
TG (mg/dl)	131 ± 53	132 ± 54	131 ± 66	127 ± 48	147 ± 60	144 ± 51
LDL-chol (mg/dl)	138 ± 38	$111\pm 35^{\dagger}$	137 ± 41	129 ± 39	136 ± 52	126 ± 44
GH dose (mg/day)	–	0.32 ± 0.22	–	0.29 ± 0.14	–	0.35 ± 0.14

* $P<0.01$ X/X vs 19/X and 19/19. $^{\dagger}P<0.01$ 1 year vs baseline. BF, body fat; FG, fasting glucose; FI, fasting insulin; T-chol, total cholesterol; TG, triglycerides.

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Table 3 Effects of rhGH during long-term (5 years) treatment in GHD adults ($n=50$) according to different *IGF1* gene promoter genotypes. Significant metabolic changes are shown in bold.

	19/19 ($n=19$)		19/X ($n=21$)		X/X ($n=10$)	
	Baseline	5 years	Baseline	5 years	Baseline	5 years
BMI (kg/m^2)	28±4	28±4	27±4	27±5	29±5	30±6
BF%	32±10	27±9*	32±8	26±8*	35±3	29±5*
IGF1 (SDS)	-1.8±1.0	-0.5±1.2*	-2.0±0.8	-0.7±0.8*	-1.9±0.8	-0.6±0.4*
HbA1c %	4.9±0.6	5.1±0.6	5.2±0.7	5.1±0.6	5.0±0.6	5.4±0.8
FG (mg/dl)	79±7	85±9*	79±9	80±8	82±9	86±8
FI ($\mu\text{U/ml}$)	9±5	10±6	12±9	8±10	12±5	14±9
HOMA	2.2±2.2	2.2±1.2	2.3±2.0	1.6±1.9	2.4±1.1	2.8±1.5
T-chol (mg/dl)	200±47	182±37*	217±44	195±35*	206±52	195±50
HDL-chol (mg/dl)	47±13	46±9	47±15	51±11	45±13	46±10
TG (mg/dl)	137±70	144±49	128±69	128±66	158±69	146±59
LDL-chol (mg/dl)	127±39	112±34*	145±42	109±56*	130±51	117±36
GH dose (mg/day)	–	0.36±0.21	–	0.35±0.13	–	0.37±0.13

* $P<0.01$ 5 years vs baseline. BF, body fat; FG, fasting glucose; FI, fasting insulin; T-chol, total cholesterol; TG, triglycerides.

Discussion

In this study, we investigated for the first time the impact of the IGF1 promoter polymorphism on the metabolic response to rhGH in a cohort of adult GHD patients. The allelic distribution was similar in GHD patients and in controls, the 19(CA) repeats allele being the most frequent, as previously reported. For this reason, patients were divided according to the presence or absence of the 19(CA) allele.

In our series of GHD adults, both the presence or absence of the WT allele and the length of the microsatellite (number of CA repeats) did not influence the response to rhGH, including increase in IGF1 levels. Independent from rhGH replacement, noncarriers of the WT allele seem to have a less favorable metabolic profile. In fact, in the X/X group, apart from a higher BF%, a significantly higher prevalence of MS was also observed, both at baseline and during follow-up. This finding does not seem to be influenced either by rhGH replacement or by severity of GHD (no difference in GH peak at stimulation test) or by primary pituitary pathology (no higher prevalence of craniopharyngioma in the noncarrier group). Even though the relatively small number of patients in each group does not allow us to draw consistent conclusions on this topic, the result of a less favorable metabolic profile in noncarriers of the WT allele is in agreement with previous studies in far larger cohorts reporting a higher prevalence of type 2 diabetes and an increased risk of fat accumulation in this genotype (10, 28).

Results obtained from longitudinal analysis of rhGH effects within each genotype group suggest that patients carrying the WT allele are more sensitive to rhGH effects

both in terms of improvement in lipid profile and worsening of insulin sensitivity, even though no difference in IGF1 levels, either at baseline or during short- and long-term rhGH therapy, was found among the three genotype-groups. Linear multiple regression analyses excluded an eventual influence of possible confounders such as sex, age at diagnosis, and presence of MS and BF%.

In the last years, many studies evaluated the relationship between IGF1 promoter polymorphism and IGF1 levels, with conflicting results. The first important study on a large population carried out by Vaessen *et al.* (10) suggested that the presence of alleles 192 (19 CA repeats) or 194 (20 CA repeats) was linked to higher IGF1 levels. On the contrary, studies performed in the following years failed to find a relationship between IGF1 levels and number of CA repeats in the promoter region (11, 12) or showed an inverse correlation (13, 14, 15). Similarly, Rosen *et al.* (16) found that low serum IGF1 levels in men with idiopathic osteoporosis were associated with homozygosity for the 192/192 allele.

The precise role of this polymorphism remains to be clarified. This particular microsatellite is located at a region known to contain specific regulatory elements of the *IGF1* gene. Some authors have speculated that allelic variation in this region might lead to changes in the promoter activity altering IGF1 transcription (29) or might be in linkage disequilibrium with another sequence in the promoter region, leading to message stability or circulating IGF1 alterations (30).

Indeed, all these studies have been performed in the attempt to find a correlation between IGF1 biochemical and genetic profile and cardiovascular or neoplastic

diseases, thus excluding subjects with pathologies regarding GH-IGF1 axis.

Some studies have been performed either in children born small for gestational age (SGA) or affected with GHD, in the attempt to find a relationship among *IGF1* gene polymorphism and catch-up growth, response to GH therapy, or IGF1 levels. Regarding children born SGA, Arends *et al.* (31) reported an association between the polymorphism of the *IGF1* gene and IGF1 levels (lower in subjects carrying the 191 bp allele). However, as underlined in a more recent review by Ester & Hokken-Koelega (32), among studies investigating an association between the 19(CA) repeat allele and prenatal growth, birth size and postnatal growth in SGA, results were conflicting, mostly due to differences in selected populations, data acquisition, and data analysis. As far as children with severe isolated GHD, a recent study has shown that homozygosity for the allele with 19(CA) repeats was associated with a less favorable short- and long-term growth response to rhGH therapy, when compared with other IGF1 genotypes, though no correlation was found between IGF1 serum level and IGF1 genotype (33). By contrast, in the same year, Miletta *et al.* (34) reported no difference among the various genotypes in rhGH effects in term of final heights.

Till now, only two studies have been performed on this topic in adults with acromegaly (17) and GHD (18), the former reporting an association between IGF1 levels and a particular IGF1 promoter genotype, the latter negating any correlation with serum IGF1 levels or rhGH replacement dose. Regarding the impact of this polymorphism on serum IGF1 levels, our results are in agreement with those reported by Meyer *et al.* (18) in 133 GHD adults. In this study, authors analyzed by genotype GH-dose after 1 year, IGF1 and IGF1 SDS values, IGF1:GH ratio, and anthropometric data. Concerning the CA repeat promoter polymorphism, no significant difference in GH doses and IGF1 concentration by IGF1 genotype was observed. To the best of our knowledge the work by Meyer *et al.* is the only report on this topic in GHD adults. Thus, this study was prompted by the lack of data on this issue in GHD adults, along with the conflicting results of previous studies carried out in different populations and, at last but not least, by the still unexplained extreme variability in the response to rhGH therapy observed in adult patients.

As previously reported by our group for the GHR polymorphism (9), present results indicate that rhGH replacement may influence at least some metabolic parameters typical of GHD adult syndrome in a slightly

different manner, according to the different IGF1 promoter genotype, with the improvement in lipid profile and the worsening of insulin sensitivity being more pronounced in patients carrying the 19(CA) allele. However, the main question arising from the present data is how this polymorphism may play a role in the metabolic response to rhGH, without exerting any impact in IGF1 serum levels.

Definitely, circulating IGF1 concentrations do not reflect the actual concentrations at local tissues, and it is tempting to speculate that local IGF1 production and its paracrine effect, not mirrored by the hepatic endocrine secretion, might mediate GH action in specific tissues such as bone, muscle, or adipose tissues. On this connection, it is possible that the IGF1 promoter polymorphism, altering IGF1 transcription or half-life, may alter IGF1 production at a local level, thus contributing to mediate GH metabolic effects in a different manner, according to a different genotype. This hypothesis might explain the impact of this polymorphism in the metabolic response to rhGH, observed in our cohort of GHD adults, in terms of improvement of lipid profile and worsening of insulin resistance, enhanced in patients carrying the WT allele.

In conclusion, the present data suggest that the IGF1 promoter genotype, which does not seem to play any role in the determination of the inter-individual variability of rhGH effects in terms of circulating IGF1 levels, may influence the metabolic response to replacement therapy. Ongoing studies will widen the long-term group of patients and give more strength to present results. Further studies, also at a molecular level, are needed to better clarify the functional role of this polymorphism and the complex relationships between different IGF1 levels and metabolic response at tissue level.

Declaration of interest

P Beck-Peccoz received a research grant from Pfizer. The other authors have nothing to disclose.

Funding

This work was partially supported by Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico.

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Received 22 July 2013

Revised version received 31 October 2013

Accepted 11 November 2013