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Long-Term Outcomes, Genetics, and Pituitary Morphology in Patients with Isolated Growth Hormone Deficiency and Multiple Pituitary Hormone Deficiencies: A Single-Centre Experience of Four Decades of Growth Hormone Replacement

Julia Rohayem[a, e] Hendrik Drechsel[a] Bettina Tittel[b] Gabriele Hahn[c]
Roland Pfaeffle[d] Angela Huebner[a]

[a]Division of Paediatric Endocrinology and Diabetology, Children's Hospital, Technische Universität Dresden, [b]Paediatric Endocrinology, Centre for Child Health Dresden-Friedrichstadt, and [c]Division of Paediatric Radiology, Technische Universität Dresden, Dresden, [d]Department of Paediatric Endocrinology, Children's Hospital, University of Leipzig, Leipzig, and [e]Clinical Andrology, Centre of Reproductive Medicine and Andrology, University of Münster, Münster, Germany

Key Words
Isolated growth hormone deficiency · Multiple pituitary hormone deficiency · Growth hormone deficiency · Genes · Pituitary morphology

Abstract
Background: Growth hormone (GH) has been used to treat children with GH deficiency (GHD) since 1966. ***Aims:*** Using a combined retrospective and cross-sectional approach, we explored the long-term outcomes of patients with GHD, analysed factors influencing therapeutic response, determined persistence into adulthood, investigated pituitary morphology, and screened for mutations in causative genes. ***Methods:*** The files of 96 GH-deficient children were reviewed. In a subset of 50 patients, re-assessment in adulthood was performed, including GHRH-arginine testing, pituitary magnetic resonance imaging (MRI), and mutational screening for the growth hormone-1 gene *(GH1)* and the GHRH receptor gene *(GHRHR)* in isolated GHD (IGHD), and *HESX1*, *PROP1*, *POU1F1*, *LHX3*, *LHX4*, and *GLI2* in multiple pituitary hormone deficiency (MPHD) patients. ***Results:*** GH was started at a height SDS of –3.2 ± 1.4 in IGHD patients and of –4.1 ± 2.1 in MPHD patients. Relative height gain was 0.3 SDS/year, absolute gain 1.6 SDS, and 1.2/2.6 SDS in IGHD/MPHD, respectively. Mid-parental target height was reached in 77%. Initial height SDS, bone age retardation and duration of GH replacement were correlated with height SDS gain. GHD persisted into adulthood in 19 and 89% of subjects with IGHD and MPHD, respectively. In 1/42 IGHD patients a *GH1* mutation was detected; *PROP1* mutations were found in 3/7 MPHD subjects. Anterior pituitary hypoplasia, combined with posterior pituitary ectopy and pituitary stalk invisibility on MRI, was an exclusive finding in MPHD patients. ***Conclu-***

E-Mail karger@karger.com
www.karger.com/hrp

Julia Rohayem, MD
Clinical Andrology, Centre of Reproductive Medicine and Andrology
Albert-Schweitzer-Campus 1, Building D11
DE–48129 Münster (Germany)
E-Mail julia.rohayem@ukmuenster.de

sions: GH replacement successfully corrects the growth deficit in children with GHD. While the genetic aetiology remains undefined in most cases of IGHD, *PROP1* mutations constitute a major cause for MPHD. Persistence of GHD into adulthood is related to abnormal pituitary morphology.

Introduction

Pituitary-derived growth hormone (pGH) has been used to correct the growth deficit in children with GH deficiency (GHD) since 1969; recombinant GH (rGH) has been applied since 1985.

In the past, assessment of GHD persistence into adulthood after attainment of final height was not performed systematically; GH substitution was discontinued as soon as the child reached near-final height. However, according to paediatric endocrine consensus guidelines [1, 2], severe GHD constitutes an indication for lifelong substitution because GH exerts beneficial effects on bone mineral density, muscle mass, body composition and well-being. Magnetic resonance imaging (MRI) or computed tomography (CT) pituitary imaging techniques have significantly improved in the past decades, and mutation analyses of genes known to cause GHD have become available. Isolated GHD (IGHD) is either sporadic or clusters in families, suggesting a genetic aetiology. To date, mutations in the growth hormone-1 gene *(GH1)* and in the GHRH receptor gene *(GHRHR)* have been identified in IGHD patients [3]. In congenital multiple pituitary hormone deficiency (MPHD), genes encoding transcription factors, essential for pituitary differentiation including *HESX1*, *PROP1*, *POU1F1*, *LHX3*, *GLI2* and *LHX4*, may be involved [3].

Here we review data from our 40-year experience with GH treatment in order to retrospectively explore the long-term outcomes of our patients with GHD. Specifically, we investigated final height and height gain achieved by GH and analysed factors influencing therapeutic outcome, including parental height, anthropometric birth data, chronologic age, height SDS and bone age at diagnosis, age at onset of puberty, GH preparations used and duration of GH replacement. By an additional cross-sectional approach with re-assessment of a subset of patients during young adulthood, we determined final adult height and the rate of severe GHD persistence, investigated pituitary morphology by imaging, and screened for mutations within genes known to be associated with IGHD and MPHD.

Patients and Methods

The files of patients who were started on GH treatment for GHD and other pathologies between 1966 and 2000 in the Paediatric Endocrine Department of the University Children's Hospital Dresden, Germany were reviewed. One hundred and twelve patient data sets were available, 96 with complete data. Seventy-eight of these patients had been diagnosed with IGHD [18 with total IGHD, 43 with partial IGHD, 17 with neurosecretory dysfunction (NSD)] and 22 with MPHD (for definitions of these conditions, see 'Definitions of Subcategories of IGHD'). All patients with GHD were invited for re-assessment in adulthood. Fifty-six (38 male, 18 female) adult patients re-attended our outpatient clinic during 2008–2009. Fifty GHD patients, 42 with IGHD and 8 with MPHD, were clinically re-investigated.

Retrospective data analysis, combined with clinical and laboratory workup and pituitary imaging at re-assessment, was performed, including dynamic GHRH-arginine testing and mutational analyses.

Definitions of Subcategories of IGHD

The diagnosis of GHD in children with short stature for age and low serum IGF-1 and IGF-binding protein-3 concentrations had been confirmed using two standard dynamic tests, but due to the long time span, different tests (exercise test, clonidine test, insulin-induced hypoglycaemia, arginine test) and different commercial kits were used. *Partial IGHD* was diagnosed with a peak GH concentration of <10 ng/ml (450 pmol/l) but >5 ng/ml (225 pmol/l) in at least one dynamic test, in combination with a second test with similar or lower GH peaks. *Total IGHD* was diagnosed if peak GH concentrations were <5 ng/ml (225 pmol/l) in two provocation tests. *NSD* was assessed (in patients with normal GH peaks in two standard dynamic tests) via GH pulse analysis in blood samples, each continuously collected over 20 min during 10 h at nighttime (8 p.m. to 6 a.m.) using the PULSAR® software (Pulsar Process Measurement Ltd., Malvern, UK). NSD was defined as an abnormal 10-hour GH secretory profile [reduced number of GH pulses <4/night and reduced mean GH secretion (<3.6 nmol/l) due to reduced peak amplitudes].

Definition of MPHD

The term MPHD was applied for patients with more than one pituitary hormone deficiency in addition to GHD.

Retrospective Data Analysis

Past medical history included birth data, age at diagnosis of GHD, data on dynamic testing, duration of GH replacement, growth chart data, therapeutic measures concerning associated hormone deficiencies, and data on pubertal onset and parental height.

GH Dosage

GH was initiated at 0.025 mg/kg body weight per day and applied via daily subcutaneous injections (~0.18 mg/kg/week) and adapted according to IGF-1 levels and response in terms of height velocity or change in height SDS.

SDS Scores, Bone Age and Parental Target Height

For comparison with the normal population, height and weight data were quantified as SDS scores using the references from Pra-

der et al. [4]. Bone age was determined according to the method of Greulich and Pyle [5]. Parental target height (TH) was calculated using the formula TH = (maternal height + paternal height)/2 + 6.5 cm for boys and – 6.5 cm for girls, with a standard deviation (SD) of 7 cm for boys and 5.5 cm for girls [6].

Workup at Re-Assessment during Adulthood

A thorough clinical examination and unstimulated pituitary function tests were performed. Blood samples were analysed with regard to IGF-1, IGF-binding protein-3, cortisol, TSH, T_3, free T_4, LH, FSH, testosterone, estradiol and prolactin levels. If low IGF-1 levels were found (<–2 SDS), the function of the somatotrophs was additionally re-evaluated using GHRH-arginine as a standard dynamic GH stimulation test, with a cutoff of 3 ng/ml (225 pmol/l) for diagnosis of severe adult GHD. Hormones were measured using commercial chemiluminescence and immunoradiometric assays. All methods were quality-controlled and accredited according to international standards. Pituitary MRI (1.5 T, Siemens Avanto; Siemens, Munich, Germany) was performed in patients without previous imaging and in all patients with MPHD, including high-resolution pituitary imaging through the hypothalamo-pituitary region. Molecular genetic screening for mutations within the *GH1* and *GHRHR* genes was performed in subjects with IGHD. Sequence analysis of genes coding for pituitary transcription factors *(HESX1, PROP1, POU1F1, LHX3, LHX4, GLI2)* was undertaken in patients with additional pituitary hormone deficiencies, excluding patients with transfusional iron overload. Genetic testing was performed using previously published methods [7]. Sequences of primers will be provided by the authors on request.

Ethics

Informed written consent was obtained for all procedures from all patients at re-evaluation. The study was approved by the local ethics committee of the Medical Faculty, Technische Universität Dresden, Germany (approval number: EK47032008).

Statistics

Statistical analysis and drafting of figures was performed using GraphPad Prism 5.01 (GraphPad Software Inc., La Jolla, Calif., USA) and SPSS 15.0.1 (IBM SPSS Inc., New York, N.Y., USA). All results are expressed as mean ± SD unless otherwise indicated. Where normality of distribution was determined by the Kolmogorov-Smirnov test, t tests for independent samples were conducted, otherwise the Mann-Whitney U test was used. Dependence between two variables was assessed using Pearson's correlation coefficient. Significance was defined as a p value <0.05.

Results

Long-Term Outcomes of GH Replacement

Ninety-six (68 male, 28 female) patients were started on GH treatment at a height SDS of –3.2 ± 1.4 for IGHD patients (n = 75) and of –4.1 ± 2.1 for MPHD patients (n = 21). In boys (n = 63), GH was replaced over 6.0 ± 4.1 years, from age 11.2 ± 3.3 years up to age 17.5 ± 3.2 years. Girls (n = 25) were treated over 4.3 ± 2.4 years, from age 10.8 ± 2.8 years to age 15.5 ± 1.9 years. Mean relative height gain was 0.3/0.31 SDS per year of GH treatment in boys/girls, and 0.3 SDS/year in IGHD and MPHD patients, respectively. Boys and girls gained 1.6 SDS (fig. 1), IGHD patients 1.2 SDS and MPHD patients 2.6 SDS (p = 0.003), with GH replaced for 4.6 ± 2.8 years in IGHD patients and for 9.1 ± 4.9 years in MPHD patients.

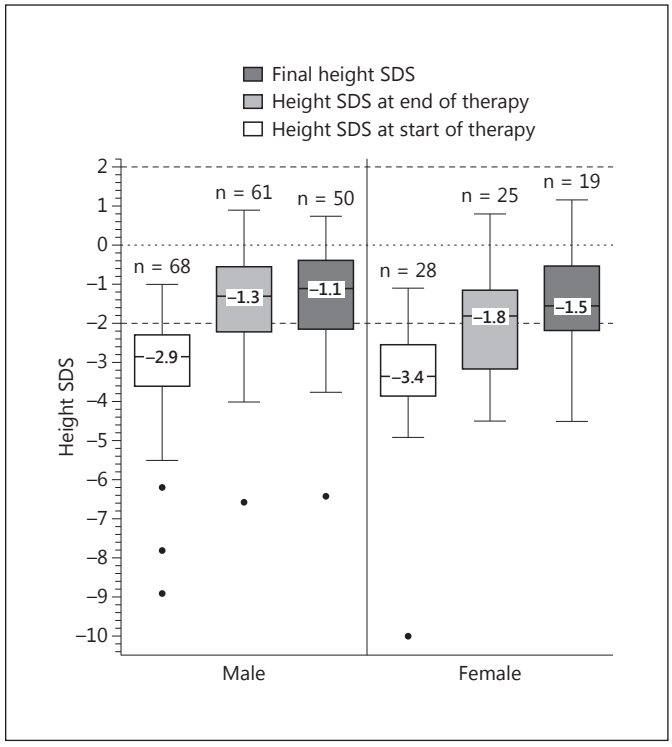

Fig. 1. Course of height SDS of boys and girls with GHD (at the start of GH therapy, at the end of replacement and final height SDS). The mean absolute height gain on GH was 1.6 SDS in boys and in girls.

Thirty-eight male and 12 female patients underwent re-assessment at a mean age of 27 years (range 19–57 years). After termination of GH substitution, boys and girls had gained a further 0.2 SDS of height on average. The outcomes in the subsets of IGHD patients, i.e. total IGHD, partial IGHD and NSD, are detailed in table 1. Males' final height was 170 ± 10 cm (–1.3 SDS), females' final height 156 ± 7 cm (–1.5 SDS). Final height was in the range of or above the individual parental TH in 54/70 (77%) patients and below it in 16/70 (23%). IGHD patients reached TH in 81% (44/54), MPHD patients in 63% (10/16) (fig. 2). The mean parental TH of GH-deficient

Table 1. GH replacement in children with IGHD and MPHD: baseline parameters and long-term outcomes

	All patients with GHD	MPHD	IGHD			Statistical test (MPHD vs. IGHD)
			total	partial	NSD	
Age at diagnosis of GHD, years (mean ± SD)						
Male	11.2±3.3	9.6±4.1	12.1±4.2	11.5±2.3	11.4±2.5	p = 0.078
	(n = 71)	(n = 16)	(n = 14)	(n = 30)	(n = 11)	Mann-Whitney U test
Female	10.8±2.8	11.3±2.9	9.4±5.5	11.2±2.4	10.4±1.7	n_{MPHD} = 21
	(n = 28)	(n = 5)	(n = 4)	(n = 13)	(n = 6)	n_{IGHD} = 78
Bone age retardation at diagnosis, years (mean ± SD)						
Male	3.3±1.6	4.2±2.1	3.2±1.5	3.0±1.0	2.6±1.4	p = 0.002
	(n = 67)	(n = 15)	(n = 14)	(n = 28)	(n = 10)	t test
Female	2.4±1.5	3.4±2.7	1.9±0.8	2.2±1.0	2.3±1.5	n_{MPHD} = 20
	(n = 26)	(n = 5)	(n = 3)	(n = 13)	(n = 5)	n_{IGHD} = 73
Height SDS at diagnosis (mean ± SD)						
Male	–3.1±1.4	–3.9±1.7	–3.2±1.9	–2.7±0.8	–2.9±0.6	p = 0.009
	(n = 68)	(n = 16)	(n = 16)	(n = 16)	(n = 16)	Mann-Whitney U test
Female	–3.4±1.5	–4.7±3.2	–3.8±0.2	–2.9±0.8	–3.2±0.7	n_{MPHD} = 21
	(n = 28)	(n = 5)	(n = 14)	(n = 28)	(n = 10)	n_{IGHD} = 75
Final height, cm (mean ± SD)						
Male	169±10	172±10	167±14	169±8	168±5	p = 0.580
	(n = 50)	(n = 11)	(n = 12)	(n = 21)	(n = 6)	t test
Female	156±7	153±11	156±6.4	158±8	154±2	n_{MPHD} = 15
	(n = 19)	(n = 4)	(n = 2)	(n = 8)	(n = 5)	n_{IGHD} = 54
Height SDS at end of therapy (mean ± SD)						
Male	–1.5±1.3	–0.9±1.5	–1.7±1.9	–1.6±1.1	–1.9±0.7	p = 0.112
	(n = 61)	(n = 14)	(n = 13)	(n = 24)	(n = 10)	Mann-Whitney U test
Female	–2.0±1.3	–2.6±1.5	–2.7±1.6	–1.5±1.3	–2.1±0.8	n_{MPHD} = 18
	(n = 25)	(n = 4)	(n = 3)	(n = 12)	(n = 6)	n_{IGHD} = 68
Final height SDS (mean ± SD)						
Male	–1.3±1.3	–1.0±1.4	–1.5±1.9	–1.3±1.1	–1.4±0.7	p = 0.883
	(n = 50)	(n = 11)	(n = 12)	(n = 21)	(n = 6)	t test
Female	–1.5±1.2	–2.0±1.9	–1.4±1.2	–1.0±1.3	–1.8±0.4	n_{MPHD} = 15
	(n = 19)	(n = 4)	(n = 2)	(n = 8)	(n = 5)	n_{IGHD} = 54
Height SDS gain during GH therapy (mean ± SD)						
Male	1.6±1.2	2.7±1.6	1.6±0.7	1.3±0.9	1.0±0.7	p = 0.006
	(n = 61)	(n = 14)	(n = 13)	(n = 24)	(n = 10)	t test
Female	1.3±1.3	2.0±2.4	1.0±1.4	1.2±1.0	1.1±0.9	n_{MPHD} = 18
	(n = 25)	(n = 4)	(n = 3)	(n = 12)	(n = 6)	n_{IGHD} = 68
Height SDS gain from start of therapy to final height (mean ± SD)						
Male	1.9±1.2	2.9±1.7	1.8±0.7	1.6±0.8	1.3±0.9	p = 0.010
	(n = 47)	(n = 11)	(n = 12)	(n = 19)	(n = 5)	t test
Female	2.0±1.3	3.5±2.2	2.3±0.9	1.7±1.1	1.5±0.7	n_{MPHD} = 14
	(n = 18)	(n = 3)	(n = 2)	(n = 8)	(n = 5)	n_{IGHD} = 51
Genetic TH, cm (mean ± SD) and percentage of patients who reached TH						
Male	173±6.2	80.7%	64.3%	83.3%	87.0%	87.5%	p < 0.001
	(n = 68)	(46/57)	(9/14)	(10/12)	(20/23)	(7/8)	Fisher's exact test
Female	159±3.7	76.2%	75.0%	50.0%	80.0%	80.0%	n_{MPHD} = 18
	(n = 28)	(16/21)	(3/4)	(1/2)	(8/10)	(4/5)	n_{IGHD} = 60

Table 1 (continued)

	All patients with GHD	MPHD	IGHD			Statistical test (MPHD vs. IGHD)
			total	partial	NSD	
Duration of GH therapy, years (mean ± SD)						
Male	6.0±4.1	9.6±5.5	5.4±3.5	4.9±2.9	4.4±2.4	$p < 0.001$
	(n = 63)	(n = 14)	(n = 12)	(n = 26)	(n = 11)	Mann-Whitney U test
Female	4.3±2.4	7.1±0.8	3.9±3.1	3.4±2.1	4.6±2.0	$n_{MPHD} = 18$
	(n = 25)	(n = 4)	(n = 3)	(n = 12)	(n = 6)	$n_{IGHD} = 70$
Start of puberty, years (mean ± SD)						
Male	14.5±2.2	15.7±2.9	15.2±2.4	13.8±1.6	13.5±1.6	$p = 0.001$
	(n = 61)	(n = 14)	(n = 12)	(n = 25)	(n = 10)	Mann-Whitney U test
Female	13.0±1.8	15.5±2.5	13.5±0.7	12.4±1.0	12.4±1.4	$n_{MPHD} = 18$
	(n = 23)	(n = 4)	(n = 3)	(n = 10)	(n = 6)	$n_{IGHD} = 66$
Persistence of GHD in adulthood						
Male	20%	89%	0%	5%	0%	$p < 0.001$
	(9/44)	(8/9)	(0/9)	(1/20)	(0/6)	Fisher's exact test
Female	0%	no patient	0%	0%	0%	$n_{MPHD} = 9$
	(0/14)		(0/1)	(0/8)	(0/5)	$n_{IGHD} = 49$

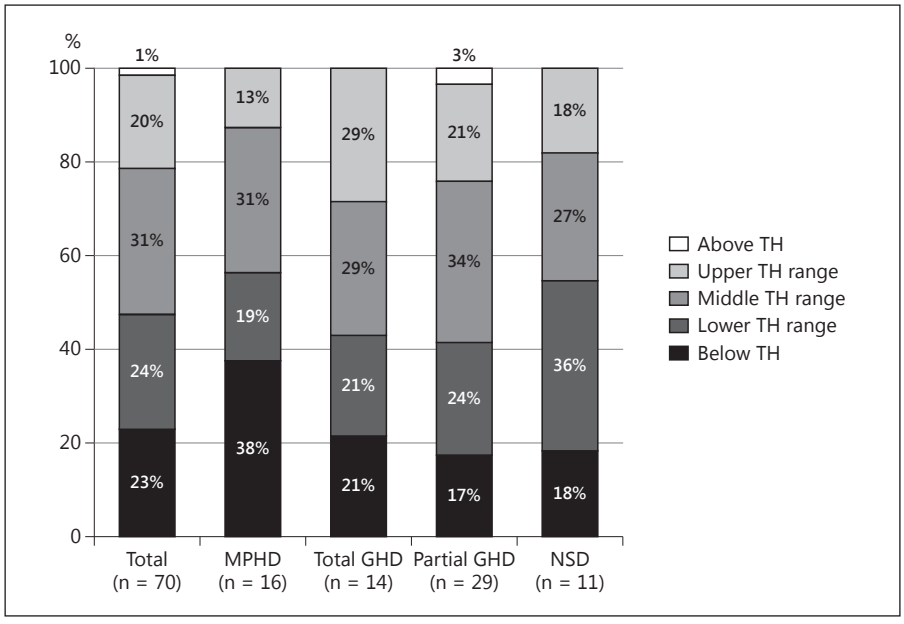

Fig. 2. Final height achieved by GH therapy in IGHD and MPHD patients in relation to parental TH.

subjects was lower than the population mean according to Reinken and van Oost [8], with a mean male TH of 173 ± 7 cm (reference 180 ± 6.5 cm) and a mean female TH of 159 ± 5.5 cm (reference 167 ± 5.5 cm).

The adult height of two patients with transfusional iron overload was within the TH range in one and below it in the other. IGF-1 levels at re-assessment were below the normal range in both.

Analysis of Factors Potentially Influencing Height Gain

Anthropometric Birth Data. The birth length and birth weight of GHD patients born at term were 50 ± 3 cm and 3,190 ± 470 g for boys (n = 55) and 48 ± 2 cm and 2,950 ± 500 g for girls (n = 25), with 23 and 14% of GH-deficient newborns having a birth length ≤–2 SDS. No significant association with final height was found ($p = 0.16$, $p = 0.92$).

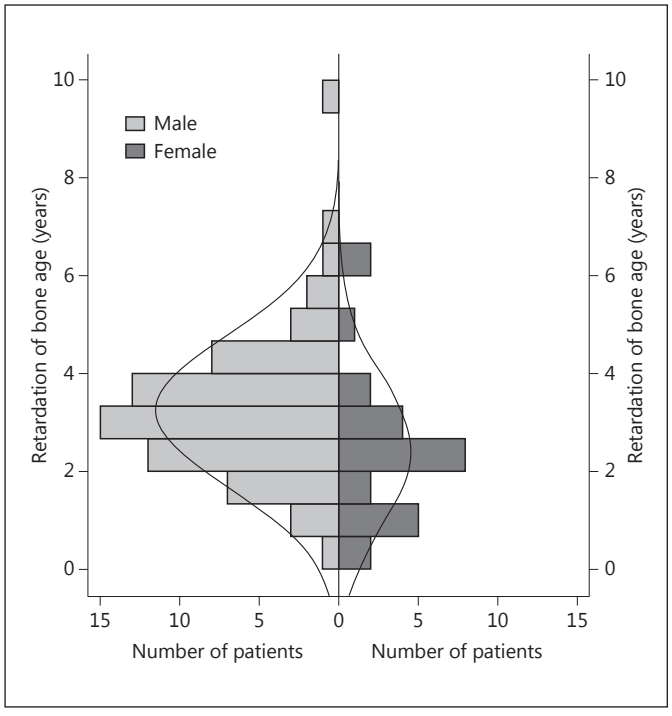

Fig. 3. Bone age retardation in male and female patients with GHD at diagnosis.

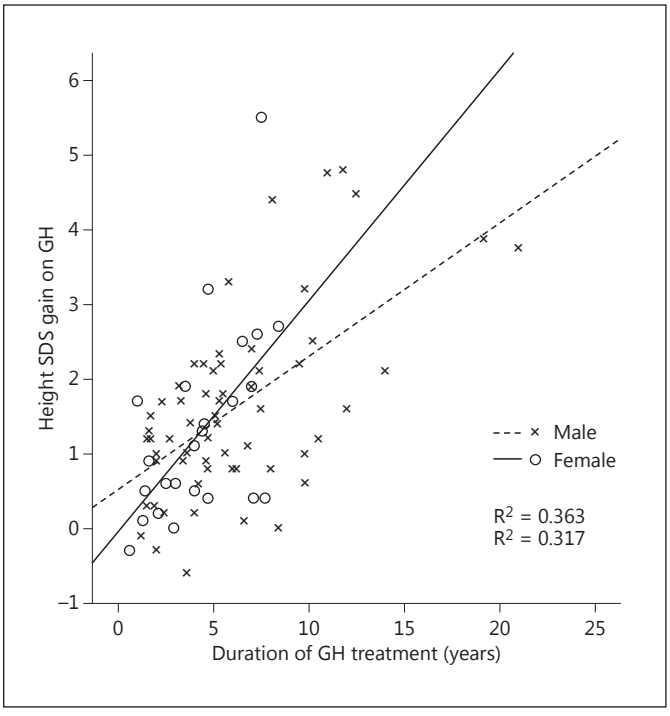

Fig. 4. Correlation of duration of GH treatment (years) and height SDS gain on GH in boys and girls with GHD.

None of the patients was large for gestational age at birth; 24% of our patients were small for gestational age (SGA).

Chronologic Age at Start of GH Replacement. No association of height SDS gain ($p = 0.183$) or final height SDS ($p = 0.633$) with chronologic age at start of GH substitution was found.

Bone Age at Start of GH Replacement. Bone age retardation was correlated with height SDS gain (Pearson's $r = 0.366$, $p = 0.001$) and final height SDS (Pearson's $r = 0.332$, $p = 0.008$). Mean bone age retardation was 3.1 ± 1.5 years in male and 2.4 ± 1.5 years in female patients (fig. 3) and 4.0 ± 2.2 years in MPHD vs. 2.8 ± 1.2 years in IGHD patients at the time of GHD diagnosis. The bone ages of the IGHD subgroups are detailed in table 1.

Height SDS at the Start of GH Replacement. Height SDS at the start of GH replacement correlated with height SDS gain (Pearson's $r = -0.492$, $p < 0.001$, $n = 86$) and with final height (Pearson's $r = 0.571$, $p < 0.00$, $n = 66$).

Duration of GH Substitution. A correlation was found between duration of GH therapy and height SDS gained by GH substitution in both male patients (R^2linear = 0.36, Pearson's $r = 0.60$, $p < 0.001$, $n = 60$) and female patients with GHD (R^2linear = 0.32, Pearson's $r = 0.51$, $p < 0.001$, $n = 25$) (fig. 4).

Age at Onset of Puberty. No correlation between age at onset of puberty and achievement of final height after GH therapy was found in either sex. Height SDS gain in subjects who were started on GH before pubertal onset was 1.4 ± 1.0 vs. 1.0 ± 0.6 ($p = 0.25$) in those with GH supplementation starting after pubertal onset. Spontaneous puberty occurred later in IGHD boys and girls than in the healthy population: mean age at onset of puberty (defined by testicular volumes ≥4 ml on each side) in all male subgroups of GHD was 14.5 years (vs. 12 years reported for healthy boys) [9]. Likewise, spontaneous puberty (defined by Tanner stage B2) was delayed in all female subgroups of IGHD, occurring at a mean age of 13.5 years (vs. 10.9 years in the reference population), mean age at menarche being 14.5 years (vs. 13.4 years in the reference population) [9]. Puberty started later in total IGHD than in partial IGHD patients of either sex (males with total/partial IGHD: 14.5/14.0 years; females with total/partial IGHD: 13.7/12.5 years). Puberty had to be hormonally induced in all patients with MPHD.

GH Preparations. Before 1984, patients with GHD were treated with pGH ($n = 21$), afterwards rGH was used ($n = 75$). Patients with replacement initiated before 1984 had more severe height SDS deviations from the mean of the

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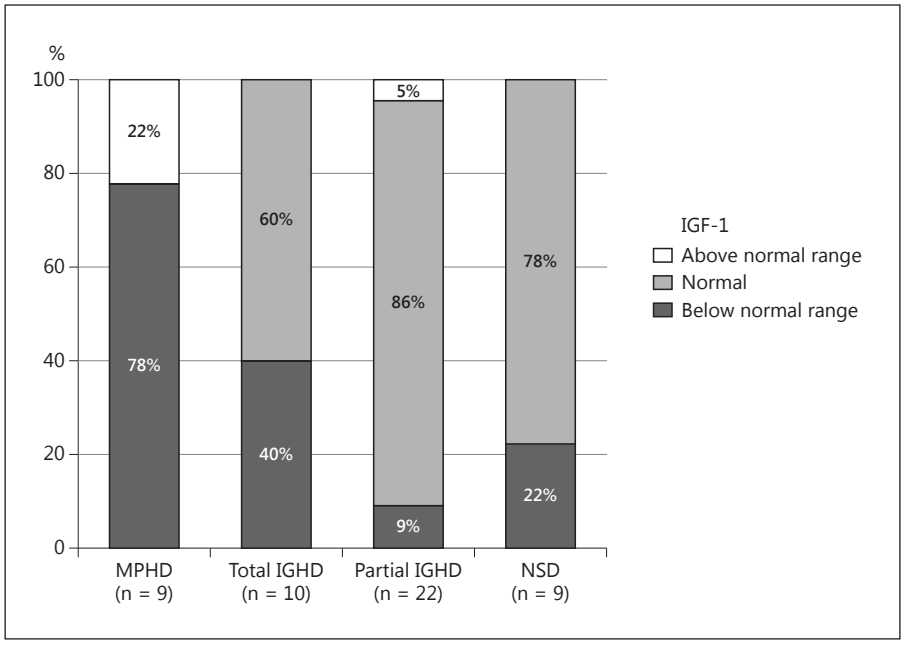

Fig. 5. IGF-1 levels of 50 GHD patients off GH therapy at re-assessment at an adult age. All patients but two at re-assessment were off GH therapy.

reference population than patients treated afterwards (median –3.6 vs. –2.8 SDS), and their bone age was more severely retarded (–4.0 ± 2.1 vs. –2.8 ± 1.3 years). Mean height gain per year was +0.35 SDS with pGH and +0.29 SDS with rGH, but with no significant differences concerning overall height SDS gain (1.2 vs. 1.4 SDS, p = 0.214).

Persistence of GHD into Adulthood

At re-assessment all but two patients with MPHD were off GH treatment since they had reached adult height. Eighty-nine percent of the patients and, among these, 55% of the subjects with MPHD had not consulted endocrine services during adulthood. Low IGF-1 levels (<–2 SDS) at re-assessment were found in 7/9 MPHD patients; two subjects had levels in the normal range because of ongoing GH replacement. In patients with previous total IGHD, 40% had decreased IGF-1 levels, whereas patients with previous partial IGHD and patients with former NSD had decreased levels in 22 and 9%, respectively (fig. 5). Only 47% of patients with low IGF-1 levels had an insufficient rise of GH levels after GHRH-arginine stimulation. Considering the results of 18 additional patients with previously performed GH stimulation tests at an adult age, overall severe GHD persistence rates into adulthood were 19% (9/47) in the IGHD cohort (22% in patients with total IGHD, 5% in those with partial IGHD and 0% in those with NSD). In contrast, GHD persisted in 8/9 (89%) of the subjects with MPHD.

Prevalence of Associated Hormone Deficiencies

Out of 7 MPHD patients without iron overload, 6 (86%) had LH/FSH deficiency, 6 (86%) TSH deficiency and 3 (43%) prolactin deficiency. ACTH secretion was compromised in 3 (43%) patients. All 3 MPHD patients with *PROP1* mutations were affected by GH, LH/FSH and TSH deficiency, 2/3 by additional prolactin deficiency and 1/3 by ACTH deficiency. In two patients with transfusional iron overload, primary hypothyroidism and secondary hypogonadism developed during adolescence, 'bronze' diabetes and hypoparathyroidism during early adulthood. None of the patients with IGHD had developed further pituitary deficiencies during adulthood.

Genetic Screening Results

In one of 41 IGHD patients (2%) a *GH1* mutation was detected, whereas *PROP1* mutations were found to be associated with MPHD in 3/7 (43%) patients. The mutation associated with IGHD was a heterozygous splice site mutation (c.IVS3 + 5A→G) in the *GH1* gene, compatible with the phenotype of IGHD type II. In three MPHD patients from two families the mutation c.150delA was detected in *PROP1*, resulting in a shift of the reading frame and a premature stop in position 109 (p.Ser109Ter). In one patient, this mutation was homozygous. In two brothers, this mutation was associated with a 2-bp deletion c.296delGA (c.301_302delGA).

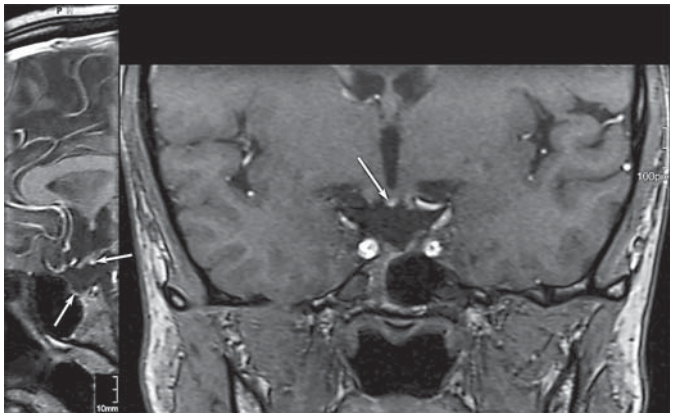

Fig. 6. Pituitary morphology on MRI of a patient with MPHD of unexplained genetic cause: An ectopic posterior pituitary gland (bright spot), a hypoplastic anterior pituitary gland with only a rim of pituitary tissue and an invisible pituitary stalk are seen.

Pituitary MRI

In two MPHD patients siderosis of the pituitary due to iron overload was visualised. The anterior pituitary gland was hypoplastic on MRI in the other 5/7 (71%) MPHD subjects, the posterior pituitary was ectopic in 3/7 (43%) and the stalk invisible in 5/7 (71%) (fig. 6). The anterior pituitary was small in 7/40 (18%) available imaging results of IGHD patients, whereas the posterior pituitary gland was eutopic with a normal pituitary stalk.

Discussion

The results of our study confirm previous findings that GH replacement during childhood is effective in normalising linear growth. The absolute height SDS gain of 1.6 ± 0.8 observed in our GHD patients and the final height SDS of –1.4 ± 1.2 were similar to the results of a large French GHD cohort [10] with a height SDS gain of 1.1 ± 0.9 and a final height SDS of –1.6 ± 0.9, and very similar to the results of a small Belgian study [11]. The mean final height SDS of our GHD boys (–1.3 ± 1.3) was identical to that of boys in a large US cohort [12] who had a final height SDS of –1.3 ± 1.0. The girls of both cohorts had similar final height SDS (–1.5 ± 1.2 in ours vs. –1.6 ± 0.9 in theirs). When differentiating between MPHD and IGHD, our medians for height SDS immediately after stopping GH treatment were higher, with –0.4 SDS for MPHD boys compared to –0.7 SDS for Caucasian MPHD boys in the large international KIGS database [13]. The median height SDS immediately after stopping GH substitution in our IGHD patients was lower than that of the Caucasian KIGS cohort (–1.5/–1.8 in our boys/girls vs. –0.8/–1.0 in KIGS), which can be explained by more severe initial height SDS deviations from the reference mean in our patients. However, their median overall height gain on GH replacement was similar to that of KIGS patients (1.7 vs. 1.6 SDS). The strength of our study compared to KIGS, which analysed near-final height data, is that we assessed definite final height during adulthood. Interestingly, we observed that a large subset of patients experienced further growth after termination of GH therapy. Considering the finding of delayed puberty in some of our IGHD patients, this may indicate that individuals with constitutional delay of growth and puberty (CDGP) could have been diagnosed with GHD as a result of GH axis immaturity at the time of standard dynamic testing. These patients may not have been categorised as GH-deficient if priming with oestrogens or testosterone had been performed. However, the recommendation for priming with sex steroids in prepubertal subjects before assessment of GH response was included in guidelines only after the mid-nineties [14, 15].

Our finding of compromised final height in comparison to mid-parental expectations is in line with previous study results: final height in our patients was in the range of or above the individual parental TH in only 77% of our patients (63% in MPHD, 81% in IGHD). In previous studies, the difference between final height and TH was –0.2 SDS in boys and –0.5 SDS in girls [13], while 89% of IGHD patients and 81% of MPHD patients achieved their genetic height potential [16]. Unphysiologic hormone supplementation or poor adherence to treatment may have contributed to lower rates of achievement of parental TH in MPHD patients.

The parents of our GHD patients were shorter than the population mean, indicating that final height in our GHD patient cohort was also influenced by familial short stature.

Height SDS and bone age retardation at baseline and duration of GH replacement have a significant influence on height SDS gain and final height, confirming previous findings [13, 17, 18] and emphasising the importance of initiating GH substitution at a young age and providing continuous therapy over a long time.

Surprisingly, no influence on height SDS gain and final height was found for age at GH start. Likewise, no influence was found for anthropometric birth data, onset of puberty or use of pGH vs. rGH. The high rate of patients born SGA in our cohort (24%), compared to a reported incidence of 5.5% among newborns born at term, may

interfere with our analysis of the impact of birth data on outcomes of GH replacement, since different growth patterns have been described for SGA children [19]. The lacking influence of the type of GH used for replacement on height SDS gain, despite lower mean final height SDS in the pGH group, is in line with previous findings [20]. Due to restricted availability of pGH, stricter criteria for GH replacement were applied, leading to a bias of selection of patients with more important height deviations from the reference population at baseline.

Our study reveals that persistent GHD was an underdiagnosed condition in adults treated for GHD during childhood and that transitional care is essential for this patient group. Nevertheless, it is surprising how few of the patients in the IGHD cohort had persistent GHD: severe GHD constituting an indication for lifelong substitution [21] was still present in adulthood in 19% of total IGHD patients and in 89% of subjects with MPHD, resulting in total persistence rates of GHD into adulthood of 27% in males and 21% in females. In previous studies, these rates varied between 33 and 80% [22–25], with one group [26] reporting rates similar to ours. Whether these relatively low deficiency persistence rates in IGHD patients indicate that GH is transient in most cases, or whether it indicates overtreatment in patients with an unknown intrinsic growth potential that is not reflected by dynamic GH tests, remains unresolved. We acknowledge that the cutoff values used in our study are arbitrary [27] due to the lack of any 'gold standard' test for GHD diagnosis, and that this problem continues to be unresolved, as GHD is a continuum between normality and abnormality.

In addition, our study underscores that in the majority of patients with sporadic IGHD, the aetiology remains unknown. Four familial forms of IGHD have been described: autosomal recessive types IA and IB, autosomal dominant type II, and X-linked recessive type III. We identified a heterozygous mutation of the *GH1* gene in only 1 out of 42 IGHD patients; no mutations were found in the *GHRHR* gene in our patients with IGHD. The *GH1* gene is located on chromosome 17q22–24, consisting of four introns and five exons. The prevalence of mutations or deletions in *GH1* is reported to be 12% in patients with more severe growth failure and at a younger age [28]. The mutation (c.IVS3 + 5A→G) in our patient causes defective splicing of the *GH1* mRNA, resulting in a GH isoform that is retained in the endoplasmic reticulum and impairs GH and other hormonal trafficking [3]. Fitting the diagnostic criteria of IGHD type II, our patient was remarkable because of his height SDS of –7.1 at diagnosis at age 15 years and his final height of 132 cm (–6.6 SDS), due to delayed pGH treatment from age 16 years until final height at age 21 years (with a height SDS gain of only 0.5) and delayed puberty occurring at age 20. The non-consanguineous parents of our patient had normal height (175/165 cm), indicating a de novo mutation. GHD persisted into adulthood, but had not been replaced. Variability in the evolution of additional endocrinopathies in patients with type II GHD has been described [3]. Our patient did not develop any other hormonal derangements until re-assessment at age 55 years.

Mutations in *GHRHR* causing type IB GHD are recognised as a significant cause of familial GHD in the Indian subcontinent. This may explain why no such mutation was identified in our German cohort.

In contrast to the low detection rates in IGHD, in patients with MPHD of our cohort, we identified mutations in the *PROP1* gene in 43%. No mutations in genes coding for other transcription factors involved in anterior pituitary development were found, leaving 57% of our MPHD patients with unknown aetiology.

The *PROP1* gene is a 'paired'-like homeobox gene located on chromosome 5q35.3 which encodes the transcription factor PROP-1. *PROP1* (acronym for 'prophet of Pit-1') is required for the expression of *PIT1* (POU1F1). Pit-1-dependent cell lines in the anterior pituitary include somatotrophs, lactotrophs and thyrotrophs and variably gonadotrophs and corticotrophs [29]. Both *PROP1* mutations found in our cohort [c.296delGA (c.301_302delGA) and c.150delA] induce a shift in the reading frame and a premature stop codon at the amino acid serine in position 107. This results in severely reduced synthesis of the encoded protein and explains the hormonal derangements found in our patients: 3/3 were affected by GH, LH/FSH and TSH deficiency, 2/3 had prolactin and 1/3 ACTH deficiency. We observed that GH treatment near-normalised final height in patients with *PROP1* mutations, confirming the findings of Crone et al. [30]. The highest frequencies of patients affected by *PROP1* mutations were reported for East-Central and Eastern Europe [31], pointing to a founder effect. Most of the mutations identified so far are represented by just two gene variants: c.296delGA (previously referred to as c.301_302delGA) and c.150delA [32]. These typical mutations were also found in all three patients with *PROP1* changes in our cohort.

Imaging data showed anterior pituitary gland hypoplasia in all MPHD subjects and an ectopic posterior pituitary in 43%, with an interrupted pituitary stalk in 71%. Comparable morphologies were described by Zimmer-

mann et al. [33] and Acharya et al. [34], with 79/43/43% and 82/65/94% of the above-mentioned changes. Initial MRI in patients with *PROP1* mutations may reveal an enlarged pituitary, which usually evolves to hypoplasia over time [35, 36]. Our MRI results indicate that at an adult age, hypoplasia of the anterior pituitary gland is the corresponding morphologic change on MRI in patients with *PROP1* defects. In contrast, the anterior pituitary was small in only 7/40 (18%) of IGHD patients, without any other abnormalities. In line with this, other groups [33, 34, 37] have reported reduced pituitary size in IGHD patients in 26, 53 and 80%, respectively.

In two patients, transfusional iron overload with siderosis of the pituitary was found to be the cause of short stature and additional endocrine failures. These patients suffered from Diamond-Blackfan anaemia. We hypothesise that their longitudinal growth was not only impaired at the pituitary level, with compromised GH secretion, but also at the hepatic level, with reduced IGF-1 responses to GH replacement.

Summary and Conclusions

Continuous GH replacement performed for 5 years in IGHD and for 10 years in MPHD was effective in normalising linear growth in children with GHD. Final heights in the range of mid-parental expectations were attained in around 80%, with an absolute height gain of +1.6 SDS and a relative gain of 0.3 SDS per year of GH substitution. Lower parental TH in GH-deficient patients points to a contribution of familial short stature. Delayed puberty and continued growth after GH termination may indicate that GHD and CDGP are overlapping conditions, potentially leading to the misdiagnosis of CDGP as GHD. This underlines the need for priming with sex steroids before carrying out dynamic testing of GH response in children of peripubertal age. While the genetic origin of IGHD remains unidentified in the majority of cases, *PROP1* mutations should be considered in subjects with combined pituitary hormone deficiencies. Hypoplasia of the anterior pituitary gland on MRI is found in both IGHD and MPHD; however, if combined with pituitary stalk interruption or ectopy of the posterior pituitary, evolving multiple endocrine deficiencies and GHD persistence are probable. While IGHD patients are not prone to develop further pituitary deficiencies during adulthood and have variable rates of GHD persistence, most MPHD patients require GH replacement during adulthood. Thorough investigation of all GHD patients at transition into adult care seems advisable to ensure continuous and lifelong follow-up of patients with hormone deficiencies.

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Disclosure Statement

The authors have nothing to disclose.

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