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ORIGINAL ARTICLE

Early retesting by GHRH + arginine test shows normal GH response in most children with idiopathic GH deficiency

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Abstract
Purpose Most children with idiopathic isolated GH deficiency (IGHD) normalize GH response to stimulation tests when retested at the completion of growth. The objective of this study was to test the effectiveness of early retesting in challenging the diagnosis of idiopathic IGHD and critically review the diagnostic workup leading to this diagnosis in children with short stature.
Methods We cross-sectionally retested 38 children with idiopathic IGHD and still on GH treatment. The initial diagnosis of idiopathic IGHD was based on subnormal GH responses to two stimulation tests and normal brain imaging or minor/nonspecific findings at magnetic resonance. The GH response normalization at retesting was considered as the main outcome measure. Clinical features of children who were falsely classified as idiopathic IGHD based on first GH testing were retrospectively analyzed.
Results GH secretion was normal in 36/38 children (95 %). Two children showed slightly reduced peak GH responses and normal IGF-I levels. Fourteen children underwent GH retesting before puberty, 24 children during puberty.
Conclusion The diagnostic process should be improved to minimize the rate of false positive at GH testing and, in case of unsatisfactory response to GH treatment, the diagnosis of isolated idiopathic GHD should be challenged with early retesting.

Keywords GH deficiency · GH therapy · Growth hormone secretion · Retesting

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Introduction

The diagnosis of GH deficiency (GHD) is not straightforward in childhood and adolescence, requiring a comprehensive clinical, anthropometric, biochemical, endocrine, and neuro-radiological assessment [1, 2]. Subnormal GH responses to stimulation tests are generally considered the mandatory prerequisite to make the diagnosis of GHD. However, pharmacological GH stimulation tests are burdened by many limitations [3]. Different assays for GH measurement yield a wide variability of GH measures [4–6]. Furthermore, GH response is affected by pubertal stage [7, 8] and body mass index (BMI) [9, 10]. Once the diagnosis of GHD has been established, therapy with daily subcutaneous injections of GH is indicated and, in the case of an appropriate first year response [11], it may be continued

until the completion of growth, when GH secretion is retested. Most children (up to 80 %) with subnormal GH responses to stimulation tests show a normalization of GH secretion when retested after the attainment of adult height [12–17]. Transient GH deficiency, poor reproducibility of GH provocative tests, false-positive GH responses secondary to pubertal delay, neuro-secretory dysfunction, improvement of hypothalamic–pituitary function after puberty, and influence of nutritional status have been proposed as potential causes of GH response recovering [18]. It is still debated whether these patients really need GH treatment until the attainment of adult height and a few data suggest that a significant proportion of patients with idiopathic isolated GHD (IGHD) show a normal GH secretion at the onset of puberty [19]. Our primary aim was to test the hypothesis that early retesting could show a normalization of GH secretion in idiopathic IGHD patients and retrospectively analyze clinical and hormonal features of children who were falsely classified as idiopathic IGHD based on first GH testing.

Subjects and methods

Subjects

Thirty-eight consecutive children (22 boys) previously diagnosed as having childhood-onset idiopathic IGHD and still on GH treatment were included. All patients were followed in the Departments of Pediatric Endocrinology at Bambino Gesù Children's Hospital and "Tor Vergata" University, Rome. The study was approved by the local ethical committees and the informed consent was obtained from all children and/or parents, after full explanation of the purpose and nature of all procedures used.

Methods

The initial suspicion of GHD was based on anthropometry. According with the criteria established by the Italian medicines agency, the diagnostic procedure for GH deficiency was started if height was less than −2 SDS and/or height velocity less than 25th centile. Weight was assessed by a digital scale, height by a Harpenden stadiometer. BMI was calculated from the ratio of weight/height^2 (kg/m^2). BMI and height were expressed as SDS, using the Italian reference data [20], and height velocity SDS was calculated using Tanner's growth charts [21]. Height and height velocity variation during GH therapy were expressed as Δheight SDS and Δheight velocity SDS (SDS 1 year after the start of GH therapy − SDS at baseline). Genetic potential for stature was evaluated by calculating target height expressed in SDS, according to the Tanner's method: boys = (mother's height + father's height + 13)/2; girls = (mother's height + father's height − 13)/2 [22]. Parental target-adjusted height SDS (height SDS − target height SDS) was calculated. Pubertal stage was assessed according to the Tanner's criteria for female breast development [23]. Tanner staging for boys was assessed by a modified genital staging method based on the average volume of both testes in males [24]. Testis volume of 1–4 ml was classified as Tanner stage I, 4–10 ml as stage II, 10–15 ml as stage III, 15–18 ml as stage IV, and ≥20 ml as stage V. GH secretion was assessed by clonidine provocative test (clonidine 100 μg/m^2 orally), arginine provocative test (arginine monohydrochloride: 0.5 g/kg given intravenously over 30 min), glucagon provocative test (30 μg/kg up to 1,000 μg intramuscularly), insulin tolerance test (regular insulin 0.1 U/kg intravenously), arginine (0.5 g/kg intravenously over 30 min) + GHRH (1 μg/kg intravenously) provocative test. In each test, blood samples for GH measurements were obtained at 0, 30, 60, 90, 120 min after the administration of the stimulus. The choice of the provocative test was based on the experience of the physicians involved in the management of cases. Priming with sex steroids was used to minimize the rate of false-positive GH responses to stimuli secondary to low circulating levels of gonadal steroids [8, 25]. Sex steroid priming was used only in prepubertal boys older than 10 years and prepubertal girls older than 9 years. Prepubertal boys were primed with 125 mg testosterone administered intramuscularly 3 days before testing. Prepubertal girls were primed with 50 μg/day ethinyl estradiol administered orally for three consecutive days before testing. Basal IGF-I levels were also measured. All patients underwent at least two GH stimulation tests at baseline. The diagnosis of GH deficiency was made on the basis of subnormal GH response to at least two different tests [3]: GH peak response to clonidine, arginine, glucagon or insulin <8 ng/ml; GH peak response to GHRH + arginine <20 ng/ml. The cut-off value for GHRH + arginine test is consistent with previous reports [26, 27]. The cut-off value of 8 ng/ml for the other tests has been defined according to the results of in-house validation performed on 130 samples assayed with both old and new WHO standard. Moreover, this 8 ng/ml cut-off has been set by the Italian medicine agency for distinguishing a normal from a subnormal GH response. We have also set the same cut-off value (8 ng/ml) for tests performed with the old standard (before 2010) to be more conservative for making the diagnosis of GHD. Multiple pituitary deficiencies were investigated by TSH, FT4, morning ACTH and cortisol measurements. LH, FSH and gonadal steroids were also measured in children older than 10 years. Children with multiple pituitary deficiencies at baseline were excluded. Karyotype analysis was performed in all female patients to exclude Turner syndrome. Children

with history of traumatic brain injury, surgery and radiation were excluded. Brain magnetic resonance imaging (MRI) scan was performed in all subjects. Maximal anterior pituitary dimensions were determined from sagittal and coronal images. Children with brain malformations, tumors, or specific abnormalities of the hypothalamic–pituitary area associated with high likelihood of permanent GHD (such as pituitary stalk section or agenesis, undescended posterior pituitary, pituitary hypoplasia defined as maximal height for the anterior pituitary <3 mm) were excluded. Children with minor and nonspecific findings at brain MRI, such as "small" (generically defined but not measured) pituitary gland and/or partial empty sella (defined as the herniation of the subarachnoidal space into the sella turcica with reduced size of the pituitary gland) were included [28–31]. All children were treated with recombinant human GH for at least 1 year. All our eligible patients were consecutively enrolled. No selection was made for the timing of retesting. Enrolled children underwent GH retesting at different ages and pubertal stages (prepuberty, Tanner stages 2–3 of puberty, Tanner stage 4–5 of puberty), after a mean (±SD) wash out time of 9 ± 3 weeks (minimum 4 weeks). GH treatment was permanently discontinued in children showing a normal GH response at retesting. Bone age was assessed by the Greulich and Pyle method [32] and bone age delay (bone age − chronological age) was expressed in years. Near adult height attainment was defined, after the completion of puberty, as the height attained when growth velocity was <1.5 cm per year over at least 6-month observation, with bone age greater than 16 years in males and 14 years in females. Since it is already known that 80 % of subjects with childhood-onset idiopathic IGHD show a normalization of GH secretion when retested at the end of growth [12–17], subjects at near adult height were excluded.

Laboratory assays

For the entire period of the study, serum GH and IGF-I levels were measured by chemiluminescence immunoassay (Immulite 2000-DPC), using commercial kits (Siemens Healthcare Diagnostics Products—Glyn Rhonwy Llanberis, Gwynedd LL55 4EL, United Kingdom). Mean intra- and interassay coefficients of variation were 4.8 and 5 % for GH, 4.9 and 5 % for IGF-I, respectively. The sensitivity limit of IGF-I measurement was 20 ng/ml, the specificity was 1,600 ng/ml. All children were initially diagnosed and underwent GH retesting at our institutions in the time range between 2007 and 2013. In the years 2007–2010, GH kits standardized to the pituitary 1st IS 80/505 WHO standard were used. Subsequently, the WHO standard for GH measurement was changed and GH kits standardized to the recombinant 2nd IS 98/574 were used. Percentiles of IGF-I were calculated using equations recently developed in our group using quantile regression [33]. These percentiles were transformed into SDS using an inverse normal function.

Statistical analysis

Data are shown as means ± standard deviation (SD) if not differently indicated. One-way ANOVA with three groups (prepubertal, Tanner stage 2–3, Tanner stage 4–5) and Bonferroni's post hoc test were performed to analyze the differences within the categories. Two-tailed t test was used to compare children with different findings at brain MRI (normal MRI vs. "small" pituitary gland/partial empty sella). Statistic analysis was performed by SPSS software (version 17, Chicago, IL, USA). A $p < 0.05$ was considered significant.

Results

Mean age of the children at the start of GH treatment was 8.78 ± 2.4 years (age 2.16–13.1). Thirty-one children (81.6 %) had no alterations of the hypothalamic–pituitary area at MRI scan, three children showed a "small" pituitary gland (7.9 %) and 4 (10.5 %) showed a partial empty sella.

The initial GHD diagnosis was established by testing with commonly worldwide used stimuli such as clonidine and arginine provocative tests in 26 children (68.4 %); GHRH + arginine and clonidine tests in three children (7.9 %); arginine and GHRH + arginine tests in one child (2.6 %); clonidine and glucagon tests in five children (13.2 %); arginine and glucagon in one child (2.6 %) and clonidine and insulin tests in two children (5.3 %). At diagnosis, two boys underwent priming with sex steroids before GH testing. Mean GH peak after clonidine was 4.57 ± 1.7 ng/ml (0.56–7.6). Mean GH peak after arginine was 4.13 ± 2.1 ng/ml (0.17–7.44). Mean GH peak after GHRH + arginine was 8.28 ± 4.5 ng/ml (4.12–14.4). Mean GH peak after glucagon was 3.08 ± 1.9 ng/ml (1.37–6.64). GH peaks after insulin were 2.16 and 2.8 ng/ml. All children were treated with recombinant human GH for at least 1 year, with a mean duration of GH therapy of 3.36 ± 2.05 years (1.1–8.0 years). The range of GH dose was 0.17–0.23 mg/kg/week. Retesting was performed by GHRH + arginine provocative test in all children. Fourteen children (36.8 %) underwent GH retesting before puberty, two boys and one girl underwent priming with sex steroids before retesting. Twenty-four children (63.2 %) underwent retesting during puberty (12 children at Tanner stage 2–3 and 12 children at Tanner stage 4–5). At retesting, mean peak GH response to GHRH + arginine was 36.97 ± 11.49 ng/ml (13.3–55.0). GH deficiency was

confirmed in two boys (5.3 %) undergoing GH retesting during puberty. In both these subjects, GHD diagnosis had been established by clonidine and arginine tests. Peak GH responses to clonidine were 4.9 and 5.1 ng/ml, and to arginine 4.1 and 6.7 ng/ml, respectively. Peak GH responses to GHRH + arginine at retesting were slightly subnormal (14.5 and 13.3 ng/ml, respectively). Both boys had normal IGF-I levels after cessation of GH therapy (IGF-I SDS for age and gender 0.23 and 0.67, respectively) and showed no abnormalities of the hypothalamic–pituitary area at MRI scan. No child was found affected by additional pituitary defects at the time of retesting. The clinical characteristics of patients at baseline are summarized in Table 1. Predicted height velocity in the first year of treatment (cm/year) and studentized residual were calculated according to the KIGS model for growth prediction in prepubertal children with idiopathic IGHD [34, 35]. No differences were found neither between the three categories of children undergoing retesting (prepubertal, Tanner stage 2–3, Tanner stage 4–5), nor between children with normal MRI and children with "small" pituitary gland/partial empty sella.

Discussion

The results of our study demonstrate that nearly 100 % of the patients who were considered as idiopathic IGHD at first testing, had a normal GH response at retesting, independently from of the timing of retesting. Early retesting of GH secretion was performed in 14 prepubertal children, and only three of them were within the age range for sex steroid priming, according to our policy. All children who underwent retesting before puberty showed normal GH secretion. Both patients with low GH response at retesting were pubertal and showed normal IGF-I levels after discontinuation of GH therapy, thus suggesting a normal endogenous GH secretion.

Data on the optimal timing for retesting are conflicting. Loche et al. [2] demonstrated an early normalization of GH response to provocative tests in a high proportion of children with idiopathic IGHD. The authors studied 33 prepubertal children (21 boys and 12 girls) with an age range of 5.2–10 years and a GH response to two provocative tests <10 ng/ml. All children had normal hypothalamic–pituitary MRI. After 1–6 months, all children underwent retesting of GH secretion by one of the provocative tests previously used. During that time, none of the children received GH therapy or entered puberty. A GH response to conventional stimuli ≥10 ng/ml at retesting was found in 28 children (85 %), while a GH response <10 ng/ml was confirmed in five (15 %). The normalization of GH secretion was not explained by the effect of puberty, as no patient had entered puberty before reevaluation. Furthermore, a potential beneficial effect of GH therapy on endogenous GH secretion could be excluded, as no patient was treated with GH before retesting [13, 36]. The authors concluded that patients with subnormal GH responses to provocative tests but normal MRI should be reevaluated before establishing a definitive diagnosis of GHD and start them on replacement therapy. On the contrary, Thomas et al. [37] reassessed GH secretion after 1 year GH treatment in 18 children (2 with multiple pituitary defects, 16 with isolated GHD); 81 % of isolated GHD patients were confirmed to be still GH deficient after 1 year from diagnosis. The authors concluded that early retesting after short-term GH treatment is not useful to identify the patients who could stop GH therapy before the end of growth. It is noteworthy that Thomas et al. included in the study seven patients with pituitary abnormalities at MRI (five with pituitary hypoplasia, two with pituitary stalk interruption). Zucchini et al. [19] performed a prospective, open label study on 69 subjects with a diagnosis of childhood-onset IGHD. The diagnosis was made by means of arginine and L-dopa tests. Children were reevaluated by the same tests after at least 2 years of GH therapy and after the onset of puberty. At retesting, low GH secretion was confirmed in 44 subjects (63.7 %). No significant differences in height deficit at diagnosis, growth response during the first year of GH therapy, age and height at puberty onset, adult height, and IGF-I levels at retesting were found between "permanently" and "transiently" GH-deficient children. Moreover, GH therapy duration and GH peak responses at diagnosis and at retesting were correlated neither with adult height nor with the total height gain during follow-up.

It has to be pointed out that we used five different provocative tests at baseline, whereas retesting was performed with GHRH + arginine test in all children. The switch from a conventional to an enhanced stimulation test could have influenced the results. It was demonstrated that conventional GH stimulation tests fail to stimulate GH secretion in a significant proportion of normally growing children [3, 25, 38, 39]. When combined with arginine (substance that inhibits the release of somatostatin), GHRH represents the most powerful stimulus to explore pituitary GH secretion. Therefore, GHRH + arginine test is helpful in differentiating normal children from patients with GHD, although a normal GH response cannot exclude the existence of a GH hyposecretory state secondary to hypothalamic dysfunction [38]. Children showing a normal GH response but subnormal IGF-I levels should undergo a second retesting with a different stimulus, such as insulin tolerance test [39]. In our study population, mean pre-therapy IGF-I concentrations were in the low normal range. It has been reported that the specificity of IGF-I measurement is high (above 90 %), whereas sensitivity is about 70 % in diagnosing childhood-onset GHD, relatively low IGF-I levels being often observed

Table 1 Clinical characteristics at baseline of children with idiopathic IGHD

	Age (years)	Sex	Pubertal stage	Height SDS	Target-adjusted height SDS	HV SDS before diagnosis	Bone age delay (years)	IGF-I SDS	Priming	BMI SDS	GH peak 1st test	GH peak 2nd test	Δheight SDS in the 1st year of GH therapy	Predicted HV in the 1st year (cm/year) of GH therapy[b]	Observed HV in the 1st year (cm/year) of GH therapy[b]	Studentized residual[b]
1	12.9	F	P2B2	−0.9	−0.3	−3.4	−1.7	−0.5	No	0.35	7.6	14.4[a]	−0.2	6.14	4.8	–
2	7.8	M	P1G1	−1.5	−1.3	−2.1	−1.3	−1.17	No	−1.42	4.8	3.84[a]	1.1	9.66	7.9	−1.21
3	7.1	F	P1B1	−2.6	−3.3		−0.4	−1.4	No	−1.51	6.22	4.98[a]	1	9.08	10.6	−0.88
4	13.1	M	P1G1	−2.2	−1.7	−1.3	−0.7	−0.71	Yes	2.41	3.75	3.74	0.78	7.96	7	0.23
5	7.9	M	P1G1	−2.4	−1.3		−1	−0.58	No	−0.87	6.64	6.72	0.5	8.42	8.1	−0.22
6	7	F	P1B1	−1.7	−1.8	−2	−1	−1	No	0.76	4.1	4.32	0.5	9.60	8.4	−2.12
7	7.2	F	P1B1	−2.3	−1.9	−2.9	−0.6	−1.64	No	−1	1.37	6.02	0.17	8.63	7.7	−0.36
8	8.6	F	P1B1	−2.6	−1.6		−1	−1.6	No	0.26	3.9	3.12	0.35	9.50	7	−1.16
9	8.5	F	P1B1	−1.8	−1	−6.1	−1.2	−1.48	No	−0.9	5.7	4.56	0.37	8.81	7.3	−0.76
10	13	M	P1G1	−2.4	−1.5		−0.9	−1.22	Yes	0.3	7.8	6.24	0.43	7.28	7.8	−0.40
11	7	F	P1B1	−2.8	−1.5		−0.9	−2.07	No	0.3	7.35	5.88	0.42	8.90	8.2	−0.48
12	8.6	M	P1G1	−3	−1.6		0.9	−1.42	No	−0.9	4.12	4.24	0.4	9.29	7.2	−1.09
13	7.2	M	P1G1	−3.2	−2.1		−1	−1.5	No	−0.5	7.56	6.05	0.61	9.48	8.2	−1.91
14	6.2	F	P1B1	−2.2	−1.4	−1.3	−2	−1.17	No	0.21	6.86	5.49	0.39	9.39	8.6	−0.82
15	13	F	P2B2	−2	−1	−2.8	−1.4	−0.8	No	0.02	6.83	6.21	0.29	6.85	7.7	–
16	7.7	M	P1G1	−2.9	−1.7		−1.4	−1.64	No	−0.67	2.82	6.84	0.47	8.31	7.8	0.61
17	8.9	F	P1B1	−2.5	−1.3	−2.9	−0.9	−2.49	No	0.1	5.2	4.16	0.6	8.95	6.9	−1.34
18	6.4	M	P1G1	−1.9	−1.7	−2.6	−1.6	−1.44	No	−0.5	7.6	6.08	0.2	9.21	6.7	−1.99
19	12.9	F	P3B2	−2.5	−1.1	−7.1	−1.2	−1.2	No	−0.72	3.6	4.64[a]	0.3	7.50	9.6	–
20	8.1	M	P1G1	−2.9	−1.2		−0.8	−2.13	No	−0.9	7.28	6.36	0.7	8.67	8.1	−1.56
21	7.6	M	P1G1	−2.4	−1.5	−1.5	−1	−1.89	No	−0.9	7.8	5.76	0.69	9.48	9.2	0.77
22	9.8	M	P1G1	−2	−1.1	−2.8	−1	−1.22	No	0.5	4.91	4.86	0.7	8.22	8	−0.29
23	13	F	P1B1	−2.6	−1.6		−1.2	−2.07	Yes	−0.9	6.9	5.52	0.2	6.94	7	0.72
24	8.4	M	P1G1	−1.9	−0.1	−6.5	−2	−0.03	No	1.96	6.1	4.88	0.28	8.69	7.8	0.62
25	7.8	M	P1G1	−2.1	−1.5	−1.2	−1	−0.13	No	−0.3	4.64	3.71	0.3	9.69	7.1	−1.50
26	8.9	F	P1B1	−2.7	−1.1	−3.4	−1.3	−2.13	No	−0.36	2.16	4.59	0.7	8.51	8.3	−0.15
27	7.5	M	P1G1	−2.6	−1.3		−0.9	−1.89	No	−0.36	6.9	5.52	0.65	9.02	6.3	−1.18
28	8.4	M	P1G1	−2.8	−1.6	−3.7	−1.2	−0.69	No	0.22	4.62	3.70	0.3	9.18	6	−0.53
29	8.5	M	P1G1	−2.4	−1.5		−1.4	−1.65	No	−0.27	7.37	5.90	0.42	8.44	6.7	−0.85
30	8.4	M	P1G1	−1.9	−1.8	−3.2	−0.8	−1.82	No	−0.4	5.78	4.62	0.39	9.07	7.5	−1.28
31	12	F	P3B2	−1.7	−1	−1.9	−0.9	−0.5	No	−0.46	7.44	11.36	0.4	6.39	7.9	–

Table 1 continued

	Age (years)	Sex	Pubertal stage	Height SDS	Target-adjusted height SDS	HV SDS before diagnosis	Bone age delay (years)	IGF-I SDS	Priming	BMI SDS	GH peak 1st test	GH peak 2nd test	Δheight SDS in the 1st year of GH therapy	Predicted HV in the 1st year (cm/year) of GH therapy[b]	Observed HV in the 1st year (cm/year) of GH therapy[b]	Studentized residual[b]
32	11.9	F	P2B2	−2.8	−2.1	−4.6	−2	−0.74	No	−0.07	0.56	6.77	0.2	7.66	8.3	–
33	9.5	M	P1G1	−2.6	−1.6		−2.3	−1.26	No	−1.44	0.17	6.38	0.19	8.28	6.5	−0.26
34	7.5	M	P1G1	−2.9	−1.5		−1	−1.67	No	−0.3	7.3	5.84	0.4	8.79	8.9	−1.23
35	7.1	M	P1G1	−2.6	−1.4	−1.9	−1.2	−2.33	No	0.16	5.32	4.26	0.39	9.33	7.2	−1.52
36	2.16	F	P1B1	−3.4	−1.5	−1.9	−1.1	−0.28	No	0.2	4.6	3.68	0.5	10.98	7.9	−3.41
37	8.2	M	P1G1	−2.5	−1.8	−2.9	−1	−2.33	No	−2.65	2.8	3.99	0.1	8.77	4.4	−0.59
38	7.9	M	P1G1	−2.9	−1.7	−2.2	−1.2	−1.55	No	−2.38	6.25	5.00	0.41	9.05	6.4	−1.41

HV height velocity, *Δheight SDS* height SDS at 1 year − height SDS at baseline

[a] GH peak at GHRH + arginine test

[b] KIGS growth prediction model for prepubertal children [Ref. 34, 35]

in children with idiopathic short stature [40–42]. Therefore, the finding of IGF-I concentrations within the normal range does not exclude GHD in about 30 % of patients [40] and should not be considered as an exclusion criterion for diagnosing GHD and starting GH therapy. However, IGF-I levels are invariably reduced in patients with severe GHD [1, 36–42]. Subnormal concentrations of IGF-I, especially if associated with low height velocity, strongly suggest GHD, provided that other causes of reduced IGF-I secretion, such as malnutrition, hypothyroidism, kidney failure, or poorly controlled diabetes, are ruled out [40].

In our country, the anthropometric diagnostic criteria to suspect GHD have been established by the Italian medicines agency (height < −2 SDS and/or height velocity <25th centile) and are still routinely used in most centers. A more rational workup should include bone age delay, the distance to target height SDS, and the change in height SDS over the foregoing years. A height within the target range, associated with normal height velocity, strongly suggests familial short stature. A significant bone age delay associated with normal height velocity and a history of pubertal delay in one or both parents is strongly suggestive of constitutional delay of growth and puberty. GH treatment should be started only in selected cases with high likelihood of idiopathic IGHD after extensive investigation. Nonetheless, early GH retesting should be performed after the first year of treatment, in case of unsatisfactory growth response.

All children with relatively "small" pituitary gland and/or partial empty sella at MRI showed a normal GH secretion at retesting. This finding confirms previous studies reporting that an MRI evidence of "small" anterior pituitary gland and/or partial empty sella but with normal position of posterior pituitary and normal pituitary stalk is suggestive of "transient" (or rather false positive) GHD, and patients with such characteristics should be reevaluated before the attainment of adult height [28–31]. Blum et al. [43] analyzed 5,805 children with idiopathic IGHD, enrolled in the multinational observational *Genetics and Neuroendocrinology of Short Stature International Study (GeNeSis)*. Multiple pituitary hormone deficiencies (MPHD) developed in 118/5,805 (2.0 %) children during follow-up. Congenital anomalies and perinatal adverse events were more common in children who developed MPHD. The definition of idiopathic IGHD was based on the information reported by the involved physicians. Children with idiopathic IGHD either did not undergo hypothalamic–pituitary MRI or had normal MRI findings, and the proportion of children with normal MRI is not reported. In our study, all children underwent brain MRI and the definition of IGHD was based on the absence of specific hypothalamic–pituitary abnormalities. Children with MPHD at initial GHD diagnosis or during follow-up were excluded, and no child was found affected

by additional pituitary defects at retesting. Though our sample size is small, the results suggest that in children identified as IGHD by more rigorous methods, the development of MPHD may be uncommon. The development of additional pituitary defects during follow-up, in a child with idiopathic IGHD, should suggest the relatively rare genetic forms of GHD and prompt molecular analyses.

In conclusion, our data support previous findings indicating that the normalization of GH secretion may occur during childhood. The high rate of normalization at early retesting is likely due to the diagnostic inaccuracy of the current available GH provocative tests that are burdened by a high rate of false-positive responses. The diagnostic workup in children referred for short stature should be based on a comprehensive approach including clinical, anthropometric, biochemical, endocrine and radiological data. GH testing should be performed only in children with a high likelihood of GHD. The risk of false-positive results to testing should be minimized by using sex steroid priming, and by taking into account the presence of overweight in interpreting the results. Early retesting should be a part of the management of patients on GH therapy and should be considered in any child with a blunted or not sustained catch-up growth during therapy, or with clinical features questioning the diagnosis of GHD.

Conflict of interest No conflict of interest to be declared.

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