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Growth Hormone Deficiency: Diagnosis and Therapy in Children

Mauro Bozzola and Cristina Meazza

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Abstract

Short stature has been defined as a height below the 2 standard deviation for age, sex and ethnicity. Growth hormone deficiency (GHD) represents a condition characterized by reduced GH secretion, isolated or associated with other pituitary hormone deficiencies. In a child with short stature and growth deceleration, after the exclusion of other causes of growth failure, the diagnosis of GHD has to be confirmed by measurement of GH secretion after at least two stimulation tests. Patients with GHD should be treated with rhGH as soon as possible, to obtain normalization of growth and normal final height. The catch-up growth in response to rhGH therapy is maximal during the first years and could be affected by many variables, such as birth-weight, age and height at start of treatment and of puberty, and duration of treatment. Overall, rhGH is believed to be safe and significant side-effects in children are very rare, including benign intracranial hypertension, hyperglycaemia, arthralgia and myalgia. Patients with childhood onset GHD are usually retested in late adolescence to confirm the GHD persistence and to continue of GH therapy. In conclusion, the present chapter provides useful and updated information about the diagnosis, treatment and follow-up of children with GHD.

Keywords: growth hormone, growth hormone deficiency, GH substitutive therapy, children, multiple pituitary hormone deficiency

1. Introduction

Human height is regulated by interactions among different factors such as genetic predisposition, nutritional status, hormonal secretion and environmental factors. Traditionally, short stature has been defined as a height of two standard deviations (SD) below the mean of sex,

age and ethnic-matched healthy controls, and is a frequent reason for referral to paediatric endocrinologists [1, 2]. Growth hormone deficiency (GHD) is a rare but important cause of short stature with a prevalence of approximately of one in 4000 during childhood [3]. Although it is a rare condition, it is important to make a correct diagnosis in order to promptly start substitutive recombinant human (rh) GH therapy and obtain a normalization of child growth. In fact, missing a diagnosis will result in poor growth and short stature adults. On the other hand, a false positive diagnosis will lead to many years of daily subcutaneous injections and significant unnecessary expenditure.

2. Growth hormone deficiency

Growth hormone deficiency is classically defined as insufficient GH secretion that results in a decrease in the production of GH-dependent hormones and growth factors, such as insulin-like growth factor-I (IGF-I), IGF-II and their binding proteins (IGFBPs) [4].

Growth hormone deficiency may be isolated or combined with other pituitary hormone deficiencies (CPHD, combined pituitary hormone deficiency) and may be congenital or acquired [5]. Acquired GHD may be secondary to hypothalamic-pituitary damage at birth or intracranial neoplasm (i.e. craniopharyngioma), infiltrative diseases (i.e. Langerhans cell histiocytosis), infections (i.e. tuberculosis, HIV), trauma, cranial or total body irradiation (TBI) and chemotherapy.

In most cases, GHD is idiopathic and only in 20% of patients an organic cause is identified. Among idiopathic cases, abnormalities in magnetic resonance imaging (MRI) of hypothalamic-pituitary region are frequent (pituitary hypoplasia, lack of pituitary stalk, ectopic posterior pituitary) [6, 7]. In some cases of GHD, an autoimmune origin may be hypothesized based on the detection of circulating anti-pituitary antibodies directed against GH-secreting cells. Anti-pituitary antibodies have also been detected in some patients with idiopathic short stature, who subsequently showed impaired GH secretion suggestive of a particular type of acquired GHD [8].

2.1. Genetics defect in isolated GHD

Gene defects have been associated with GHD (**Table 1**). Mutations have been found in the genes encoding for GH (*GH1*) or GH releasing hormone receptor (*GHRHR*). *GH1* mutations can either lead to classic GHD (types IA, IB and II) or bio-inactive GH syndrome, a condition characterized by normal or elevated circulating not active GH levels [9]. Homozygous *GH1* deletions are a common cause of type IA GHD and patients can develop anti-GH antibodies during GH therapy. Type IB GHD is a less severe form and is caused by mutations of *GH1* or *GHRHR*, while GHD type II is a dominant form caused by skipping of exon 3 which results in the secretion of a GH isoform (17.5 kDa) with a dominant negative effect [10]. Furthermore, the X-linked type III GHD is associated with agammaglobulinemia and has been associated with mutations in *BTK* and *SOX3* genes [11, 12]. Another cause of GHD could be mutations of the gene encoding for the ghrelin receptor (*GHSR*), which decrease in GH secretion [13].

Disorder*	Gene(s)	Inheritance	Clinical features/GH levels	Response to GH therapy	References
Isolated GHD type IB (612781)	GHRHR	AR	Low serum GH	Positive	[106, 107]
Isolated GHD type IA (262400)	GH1	AR	No serum GH	Often anti-GH antibodies	[106, 107]
Isolated GHD type IB (612781)	GH1	AR	Low serum GH	Positive	[106, 107]
Isolated GHD type II (173100)	GH1	AD	Variable height deficit and pituitary size; low GH levels; other pituitary deficits can develop	Positive	[106, 107]
Isolated GHD type III (307200)	BTK, SOX3	XLR	Low GH levels with agammaglobulinaemia	Positive	[106, 107]
Isolated partial GHD(615925)	GHSR	AR, AD	Variable serum GH and IGF-I	No data	[13, 108]
Almstrom syndrome (203800)	ALMS1	AR	50% of cases are GHD	No data	[16]
	RNPC3	AR	Severe GHD, hypoplasia anterior pituitary	No data	[14]
	IFT172	AR	Functional GHD, retinopathy, metaphyseal dysplasia, hypertension	Positive	[15]

*Name (number) according to OMIM.

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive.

Table 1. Gene defects associated with isolated GHD.

Finally, many studies reported isolated GHD associated with congenital syndromes caused by mutations and/or deletions of different genes not directly involved in growth (i.e. biallelic mutations in *RNPC3*, compound heterozygosity for *IFT172* or mutation of *ALMS1*) [14–16].

2.2. Genetics of combined pituitary hormone deficiency

In childhood, some patients may show GHD associated with deficiency of other pituitary hormones such as prolactin, thyroid stimulating hormone (TSH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and, sometimes, adrenocorticotrophic hormone (ACTH) [4].

Several genetic defects of transcription factors have been reported in CPHD (**Table 2**). Two categories of patients with hypopituitarism are described according to the presence or absence of extra-pituitary abnormalities and/or malformations, beside anterior pituitary hormone deficiencies [17]. The phenotypes with heterogeneous extra-pituitary abnormalities are caused by mutations in transcription factor genes early expressed in regions determining the formation of forebrain and related midline structures, such as hypothalamus and pituitary. Defects have been found in *SHH*, *FGFR1* and *FGF8*, *LHX3* and *LHX4*, *HESX1*, *SOX2* and *SOX3*, *OTX2*, *PROK2* and *PROKR2*, *PITX2* and many others [18]. On the other hand, phenotypes without any extra-pituitary malformations are due to mutations of late-acting pituitary-specific transcription factors. Mutations in transcription factors such as *POU1F-1* and *PROP-1* are commonly linked to deficiency of GH, TSH, LH, FSH and sometimes ACTH [19]. Furthermore, as recently described by Giordano et al., deletions of particular chromosome regions including these genes lead to syndromes often associated with isolated GHD or CPHD [20].

Disorder*	Gene(s)	Clinical features	Inheritance	References
CPHD-1 (613038)	POU1F1	GH, PRL, variable TSH deficiency	AR, AD	[18, 109, 110]
CPHD-2 (262600)	PROP1	GH, PRL, TSH, LH, FSH, variable ACTH deficiency	AR	[18, 109, 110]
CPHD-3 (221750)	LHX3	GH, PRL, TSH, LH, FSH deficiency	AR	[18, 109, 110]
CPHD-4 (262700)	LHX4	GH, TSH, ACTH deficiency	AD, AR	[18, 109, 110]
Septo-optic dysplasia (CPHD-5) (182230)	HESX1	Optic nerve and pituitary hypoplasia, midline abnormalities of brain, TSH, GH, PRL, LH, FSH and ACTH deficiency	AR, AD	[18, 109, 110]
CPHD-6 (613986)	OTX2	GH, TSH, LH, FSH, variable ACTH and PRL	AD	[18, 109, 110]
Axenfeld-Rieger syndrome type1 (180500)	PITX2	Brain abnormalities, variable pituitary deficiency	AD	[109]
Optic nerve hypoplasia and abnormalities of the central nervous system (206900)	SOX2	Variable GHD, LH and FSH deficiency, developmental delay	AD	[109, 110]
X-linked panhypopituitarism (312000, 300123)	SOX3	GHD or CPHD, mental retardation	XLR	[18, 109, 110]
Pellister-Hall syndrome (146510)	FGF8	Holoprosencephaly, septo-optic dysplasia, Moebius syndrome	AR	[18, 110]
	FGFR1	Pituitary and corpus callosum hypoplasia, ocular defects	AD	[18, 111]
	PROKR2	Variable pituitary hormone deficiency	AD	[111]

*Name (number) according to OMIM.

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive.

Table 2. Genetic defects reported in CPHD.

3. Diagnosis of GHD

The diagnosis of GHD in children should be based on clinical and auxological assessment, and radiological evaluation, along with biochemical tests for the pituitary-IGF axis. In some cases, genetic testing is requested.

3.1. Clinical and auxological assessment

Growth hormone deficiency can develop at any age (from the first few months of life to adolescence) and signs may vary. Generally, the neonate with isolated GHD exhibits hypoglycemia, prolonged jaundice and microphallus and/or cryptorchidism in males [4].

In pre-pubertal short children, the most common symptoms of idiopathic GHD are short stature and reduced growth velocity for age. The phenotypic characteristic features of severe GHD are immature faces, prominent forehead, depressed midline development, single central incisor, optic nerve hypoplasia, thin and sparse hair, slow nail growth, high-pitched voice, low muscle bulk, increased subcutaneous fat and low-density lipoprotein cholesterol [4].

When GHD is suspected in a child with short stature, other causes of growth failure such as hypothyroidism, chronic systemic diseases, Turner syndrome, malnutrition or skeletal disorders should first be excluded before starting endocrinological evaluation.

As recommended by the Consensus Guidelines for Diagnosis and Treatment of GHD in Childhood and Adolescence from the 2000 meeting of the GH Research Society [21], statement criteria to start evaluation for GHD are as follows:

- Severe short stature (height <3 SD below the mean);
- Height less than -1.5 SD below the mid-parental height;
- Height less than -2 SD below mean and either height velocity less than -1 SD below mean over the past year or decrease in height SD of more than 0.5 SD over the past year;
- In the absence of short stature, height velocity less than -2 SD below mean over 1 year or less than -1.5 SD below mean over 2 years;
- Signs of intracranial lesion;
- Signs of combined pituitary hormone deficiency; and
- Neonatal signs and symptoms of GHD, including hypoglycaemia, prolonged jaundice, microphallus and/or cryptorchidism, cranial midline abnormalities.

Generally, children with GHD do not show any metabolic abnormalities, notwithstanding the biological effects of GH on the different metabolisms. Recently, we reported that lipid and glucose metabolism are only slightly affected in GHD children but GH replacement therapy affects the secretion of factors such as leptin and resistin by adipose tissue [22]. Growth-hormone-deficient patients frequently show reduced bone mineralization with decreased bone density for delayed skeletal maturation. However, a study by Hogler and collaborators

demonstrated that, in GHD children, cortical and trabecular densities are normal and the risk of fracture is not increased [23]. Similarly, inflammatory cytokines, such as TNF- α , IL-6 and IL-12, are comparable between GHD children and age-matched healthy controls and are slightly influenced by a short-term rhGH therapy [24, 25].

3.2. Radiological investigations

In the child over 1 year of age, bone age is routinely estimated from an x-ray of the left wrist and hand [21]. Sometimes in 2- or 3-year-old children the results may not be accurate, bone age can be evaluated more precisely from X-rays of the knee and foot, as in the neonate. Generally, the severity and duration of GHD affects delayed bone maturation. Bone age is usually assessed using the Greulich and Pyle radiological atlas [26] and/or the Tanner and Whitehouse (the TW2) method [27].

Magnetic resonance imaging is the most frequently used technique to visualize hypothalamic-pituitary anatomy. Neuroimaging in short children may rule out a tumour, in particular a craniopharyngioma or optic nerve glioma. The most common radiological findings in GHD children are an ectopic posterior pituitary gland, anterior pituitary hypoplasia and a thin pituitary stalk, which may be also present in combination. Other abnormalities associated with GHD may be septo-optic dysplasia, corpus callosum hypoplasia and presence of empty sella. However, many idiopathic GHD children show no pituitary abnormalities and some authors demonstrated that those GHD children with MRI abnormalities have more severe short stature and younger age at diagnosis compared with those GHD patients with normal pituitary [28].

3.3. Provocative GH testing

The measurement of GH secretion and not of basal GH levels, which are normally low, is used for confirming the diagnosis of GHD. Growth hormone secretion is pulsatile throughout the 24 hours, with peaks occurring at the time of slow-wave electroencephalographic rhythm and is regulated by multiple peptides and neurotransmitters, in particular GHRH and somatostatin. Furthermore, GH secretion significantly varies with gender, age, weight and pubertal status: during puberty it markedly increases, due to increased sex steroid levels, and then it decreases with age, especially in males [29].

Different cut-off values in children, adolescents and adults have been proposed by the GH Research Society to confirm the diagnosis of GHD. However, a specific cut-off value based on age, sex, weight and pubertal stage does not exist and should be established to improve the interpretation of GH stimulation tests.

Therefore, in current clinical practice, the diagnosis of GHD relies on biochemical measurement of GH secretion after stimulation tests. Various commercially available assays for measuring GH exist and several studies have shown an inter-assay variability of GH values [30, 31] that leads to a wide discrepancy in the results obtained in different analysis laboratories. They remarkably depend on different factors, such as the assay methods and the different calibrators used, the specificity of antibodies (monoclonal or polyclonal), the matrix difference

between standards and samples and the interference with endogenous GH binding proteins (GHBP) [32].

Commercially available immunoassays may detect different GH variants present in serum, since GH circulates in many isoforms due to alternative splicing, polymerisation and complexing with other molecules [33]. Moreover, we recently reported that GH values may also depend on different calibrators (i.e. IS 98/574, a recombinant 22 kDa molecule of more than 95% purity, and IS 80/505, of pituitary origin and resembling a variety of GH isoforms) used in the same GH assay [34]. Variations in GHBP have been found to significantly affect the GH concentration detected, since in serum up to 50% of GH is bound to GHBP [35]. According to the most recent international recommendations on GH assay standardization, only the 22 kDa recombinant IS 98/574 should be used and GH results should be expressed in mass units and, since 2006, major clinical endocrinology journals accept only those manuscripts in which GH data are expressed in micrograms per litre for IS 98/574 [36, 37].

The effect of the assay variability can lead to significant differences in the diagnosis of GHD among laboratories, since different cut-off values are used. In fact, the serum GH cut-off value for GH secretion depends on the method used for determining serum GH [38]. Different immunoassays (RIA, IRMA, ELISA, chemiluminescent and immunofunctional assays) are widely used in clinical laboratories because of speed, sensitivity and convenience. However, cut-off limits of GH stimulation tests to diagnose GHD in childhood and adolescence are not sufficiently validated by clinical studies. In the last years, in clinical practice, the traditional cut-off value of 10 ng/ml, which had been validated in the 1960s and 1970s, using developed radioimmunoassay, was widely used [21]. Recently, a study by Wagner et al. established new method-specific clinical evidence-based GH cut-off limits, showing that those limits varied from 4.32 to 7.77 ng/ml for seven hGH assays [39]. Thus, the cut-off level for the diagnosis of GHD has been revised and 8 ng/ml is accepted as the cut-off limit using Immulite 2000 assay. A GH peak concentration below 4 ng/ml defines complete GHD and a peak between 4 and 8 ng/ml indicates partial GHD.

Furthermore, different cut-off points have been proposed based on children BMI and in response to GHRH+arginine test: for lean children a peak cut-off value of 11.5 ng/ml, for overweight children a peak cut-off value of 8 ng/ml and for obese children a peak cut-off value of 4.2 ng/ml [31]. However, these cut-off points have not yet been validated.

The consensus guidelines of the Growth Hormone Research Society for diagnosis and treatment of GHD in children have established that in a child with suspected isolated GHD, two stimulation tests are required, since there are large numbers of false positive diagnoses from single stimulation test in normal children [21]. On the contrary, in a child with central nervous system pathology, cranial irradiation or genetic defects only one GH stimulation test is needed [21]. Due to the lack of reproducibility and accuracy of these tests, the clinician should remember that the diagnosis of GHD is mainly based on clinical and auxological findings and that the results of the stimulation tests are only confirmatory [40].

Many different stimuli are currently used to induce GH secretion, since they act through different mechanisms. Indeed, no stimulation test is completely reliable, although for clinical

practice the Insulin Tolerance Test (ITT) is the gold standard [29]. As summarized in **Table 3**, different pharmacological stimuli are used to measure GH secretion [41]. Sometimes, to improve specificity of the test, pharmacological stimuli may be combined, for example, the combination of GHRH and arginine. These tests should be carefully monitored by an experienced team.

Stimulus	Dosage	Time samples (minutes)
Insulin tolerance test i.v.	0.05–0.01 U/kg	0, 15, 30, 45, 60 and 90
Arginine HCl i.v.	0.5 g/kg (max 40 g)	0, 30, 60, 90 and 120
Clonidine i.v.	0.15 mg/m ²	0, 30, 60 and 90
Glucagon i.m.	0.03 mg/kg (max 1 mg)	0, 30, 60, 90, 120, 150 and 180
GHRH i.v.+arginine HCl i.v.	1 µg/kg GHRH, arginine 0.5 g/kg (maximum of 40 g)	0, 15, 30, 45, 60, 90 and 120

i.v., intravenously; i.m., intramuscularly; p.o., per os.

Table 3. Currently used GH stimulation tests.

Insulin tolerance test is considered the best stimulation test, although it is risky because of the hypoglycaemia induced by insulin administration. GH secretion is stimulated by the response to insulin-induced hypoglycaemia. Usually, 0.1 unit/kg of insulin is administered intravenously (i.v.) in children over 4 years of age and 0.05 unit/kg in younger children. Blood samples for GH analysis and glucose levels should be obtained at 0, 15, 30, 45, 60 and 90 minutes after administering the insulin dose. The test is considered valid if the blood glucose level decreases by 40–50% of the initial value or reaches less than 40 mg/dl (i.e. 2.22 mmol/l). The GH peak occurs 15–30 minutes after the glucose nadir. Children with GHD frequently have an enhanced response to insulin that results in severe hypoglycaemia.

Arginine stimulates GH secretion by inhibition of somatostatin release. Arginine HCl (0.5 g/kg to a maximum of 40 g) is administered i.v. over a 30-minute period. Blood samples for GH determination should be taken at 0 (baseline), 30, 60, 90 and 120 minutes. The maximum GH peak is expected to occur at 60 minutes after starting arginine infusion. Nausea and vomiting are frequently observed side effects.

Clonidine, an alpha 2-adrenergic agonist, increases GHRH secretion and inhibits somatostatin release. Clonidine is administered at a dose of 0.15 mg/m² [42]. Samples for this GH assay should be obtained at 0 (baseline), 30, 60 and 90 minutes. GH peak is usually recorded 60 minutes after clonidine administration and is usually greater than in tests with other stimuli. After clonidine administration, blood pressure may fall, and young children may show drowsiness for several hours.

Glucagon is another very used stimuli for GH secretion, which is stimulated by endogenous insulin glucagon-induced to compensate for elevated serum glucose levels. Glucagon is administered intramuscularly (i.m.) or subcutaneously (s.c.) at a dose of 0.03 mg/kg to a maximum of 1 mg. Serum samples are obtained at 0, 30, 60, 90, 120, 150 and 180 minutes after administration. The maximal GH peak can occur 2 hours after glucagon injection. After glucagon administration young children may develop nausea and vomit.

Growth hormone releasing hormone is the endogenous stimulating factor of GH and the administration of GHRH can directly assess the capacity of the pituitary to secrete GH. Somatostatin inhibitors, pyridostigmine and arginine are used to enhance the GH response and to reduce the intra- and inter-individual variability, due to fluctuations in somatostatin secretion. The GHRH test, in combination with arginine, is a very useful tool to identify defects at the hypothalamic level, especially in children with CPHD. The GHRH+arginine test stimulates GH to a greater extent than the GHRH test alone. Growth hormone releasing hormone is given i.v. at a dose of 1 µg/kg at time 0 and arginine, at a dose of 0.5 g/kg (maximum of 40 g), i.v. infusion is given from time 0 to time 30. Serum samples for the GH analysis are obtained at 0, 15, 30, 45, 60, 90 and 120 minutes. The cut-off for this test is 20 ng/ml for the GH peak in childhood and 19.0 ng/ml in late adolescent and early adulthood [43]. Furthermore, the GHRH plus arginine test is useful for identifying false positive GHD in children showing a blunted GH response to classic stimuli in contrast with a normal growth rate [44]. Finally, GHRH+arginine test is particularly used in the re-testing of GH secretion at the end of GH therapy in childhood-onset GHD patients.

Growth hormone secretion may be evaluated by more physiologic tests, such as the exercise test, 24-h GH profiling and urinary GH estimation. Although they show minimal side effects for the patient and are less expensive than pharmacological tests, these tests are no longer used for the diagnosis of GHD in clinical practice, but they are still useful for research investigation.

GH testing in children in the peri-pubertal period and in those with delayed puberty frequently yields subnormal results due to a diagnosis of constitutional delay or, more probably, a sex steroid deficiency, since circulating levels of sex steroids increase during puberty, resulting in an increase in pulse amplitude of GH secretion, IGF-I concentration and anterior pituitary size. Therefore, the use of oestrogen or testosterone to prime the GH axis prior to pharmacological stimulation tests may facilitate GH release in pre-pubertal children and reduce false positive rates. Priming may be performed with oral oestrogen (10–20 µg ethinyloestradiol) for 3 days prior to GH stimulation test in girls or intramuscular injection of testosterone enanthate (100 mg) 7–10 days prior to stimulation test in boys [3]. However, not all paediatric endocrinologists agree that sex-hormone priming is required [45], since it only briefly augments the GH response which then returns to suboptimal concentrations and this may lead to under-diagnosis of peri-pubertal children that could have benefited from GH treatment. Some authors recommend that priming may be considered only in adolescents with pubertal delay (girls aged >11.5–12 years and boys aged >13–13.5 years) with no signs of puberty or only initial ones [46]. Therefore, at present there is no agreement on the use of priming.

3.4. Measurement of IGF-I and IGFBP-3

Because of the low reliability of the pharmacological tests, in the last years newer diagnostic procedures such as assay of IGF-I and IGFBP-3 serum levels, genetic testing and neuroimaging have been considered for confirming the diagnosis of GHD in children [47]. However, in many European countries it is yet mandatory to show a reduced GH secretion to at least two stimulation tests in order to start substitutive rhGH therapy in children.

The IGFs are GH-dependent peptides that mediate many of the anabolic and mitogenic actions of GH. The levels of IGF-I and its major binding protein IGFBP-3 greatly depend on GH secretion. Since serum levels of IGF-I are stable during the day, it should be possible to assess GH status by measuring IGF-I levels. However, most of the assays for IGF-I measurements do not show good sensitivity and specificity and are used in the diagnosis of GHD in children [48]. Furthermore, since IGF-I levels are influenced by age and pubertal development, an overlap between IGF-I values for normal and GHD children still exists, particularly in children younger than 5 years. Most investigators use cut-offs of either the fifth percentile or <-2 SD score to define subnormal levels of IGF-I [47]. Moreover, reduced IGF-I levels may occur in children with malnutrition, hypothyroidism, hepatic disease or diabetes mellitus.

Insulin-like growth factor binding protein-3 levels have also been considered for the detection of GHD and were thought to be potentially superior to measurement of IGF-I alone as IGFBP-3 is less nutritionally sensitive than IGF-I. However, many studies found no differences in IGFBP-3 levels between GHD and non-GHD subjects [49].

In conclusion, although reduced IGF-I and IGFBP-3 levels may suggest a condition of severe GHD, normal serum IGF-I and IGFBP-3 values may not allow to exclude GHD. Therefore, GH stimulation tests are widely used for confirming GHD diagnosis in children.

3.5. Genetic investigation

Genetic testing is not routinely performed in the diagnosis of GHD. However, numerous mutations leading to GHD have been identified and with the development of new genetic technologies, such as whole exome and whole genome sequencing, screening for mutations in the diagnosis of GHD may play a critical role in the coming years.

Generally, signs of GHD of genetic origin are particularly evident and may include: early onset of growth failure, positive family history, height more than 3 SD below the mean, extremely low GH response to provocation tests, very low IGF-I and IGFBP-3 levels. In these cases, genetic analysis is strongly suggested.

The most common mutations in patients with isolated GHD have been identified in *GH1*, *GHRHR* and *RNPC3* genes and may be associated with a normal MRI scan (**Table 1**). Other gene mutations (i.e. *POU1F1*, *PROP1*, *LHX3*, *LHX4*, *HESX1*, *SOX2*, *SOX3*, etc.) present in GHD, along with other pituitary deficiency, are associated with clinical and radiological features (**Table 2**).

4. Treatment of GHD children

As recommended by the GH Research Society [21], patients with proven GHD should be treated with rhGH as soon as possible after the diagnosis is made, for normalizing height during childhood and obtaining normal adult height. The response to GH therapy could be affected by variables such as birth-weight, age and height at start of treatment and at start of puberty, extent of the GHD and duration of treatment [50, 51]. The pattern of catch-up growth in GHD infants during GH therapy indicates a sustained and significant effect during the first years of treatment, followed by a progressive decrease in growth velocity, known as “waning effect” in the subsequent years [52]. Nevertheless, the growth rate is always higher than it was before starting the therapy, suggesting that GH therapy may still be advantageous for patients.

Growth hormone treatment in childhood also normalizes body composition, reducing body fat, generating a reversible insulin insensitivity, increasing the ratio of high-density lipoprotein to total cholesterol and accelerating bone remodelling with the increase of bone mineral mass [22].

Until 1985, for more than 30 years, GHD was treated by pituitary-derived GH. Then, rhGH was introduced into clinical practice and now the presently available rhGH brands for registered clinical indications are obtained by expression either in *Escherichia coli* bacteria or in mammalian cell lines, such as mouse C127. Recently, identical recombinant DNA-derived proteins have been expressed in other biological systems (i.e. *Saccharomyces cerevisiae*); these proteins have the same structure and similar profiles in terms of quality, safety and efficacy and are termed “biosimilars.”

Growth hormone should be administered s.c. in the evening on a daily basis, and the rhGH dosage should be expressed in milligrams per kilogram of body weight per day. The recommended starting dosage for rhGH is 0.025–0.05 mg/kg/day, according to a 6-day per week schedule. An increment in the dose from 0.03 to 0.07 mg/kg per day is suggested during puberty, to maximize longitudinal growth during this period of life. An alternative approach to the fixed dose is auxology-based dosing. This approach consists of starting treatment with the lowest accepted dose and then titrating upwards based on weight, growth velocity and IGF-I concentrations, still keeping the GH dose within the dose range [53]. IGF-I titration consists in adjusting the GH dose to a target IGF-I concentration irrespective of growth rate and the usual range of GH dose. This leads to the use of very high GH doses (i.e. 0.091 mg/kg/day) in order to find an improvement in growth rate in comparison with patients treated with a fixed dose [54]. Since there are very few safety data on the use of GH at such high doses, this approach cannot be recommended at the present time. Actually, the major current alternative strategy is prediction model-based dosing. Prediction models may lead to a more evidence-based approach to determine the GH dose regimen and may reduce the response variability and drug costs for GH treatment. Three types of prediction models have been described, all using auxological data. It is uncertain whether adding biochemical, genetic or proteomic markers may improve the accuracy of the prediction [55].

Subcutaneous injection (s.c.) has become the standard administration route for GH because of its ease of administration and patient acceptance. Among experimental studies on possible

other ways of delivery of GH, a multi-centre study focused its attention on the inhalator route [56]. The authors showed that GH delivered by inhalation was well tolerated and resulted in a dose-dependent increase in serum GH and IGF-I levels, suggesting that the delivery of GH via the deep lung is feasible in children and should deserve attention.

Furthermore, in order to obtain a better compliance of patients to years of daily treatment, long-acting forms of GH have been developed, utilizing different techniques: depot GH formulations, pegylated formulations, pro-drug formulations and GH fusion protein technology [57]. These formulations show different pharmacodynamic and pharmacokinetic profiles, all being effective in extending GH action and prolonging increase IGF-I concentrations. Clinical data in humans are still very limited and short-term studies showed that treatment with long-acting GH preparation is effective and safe in GHD children and adults. Many of these formulations frequently showed injection-site reactions with erythema, induration or lipodystrophy. Long-term studies are needed to confirm the value and safety of these agents [58].

The routine follow-up of GHD children should be performed by a paediatric endocrinologist on a 3- to 6-month basis. The determination of the increase in height and change in height velocity during rhGH treatment is useful in assessing the response to GH. A study from the International Growth Study database (KIGS) showed that during GH therapy it is important to monitor IGF-I levels to check the compliance of the patients and ensure that IGF-I values do not exceed the normal range, since high levels of IGF-I have been linked to the development of tumours [59]. Therefore, supra-physiological concentrations of IGF-I should be avoided [60].

If GHD is part of a combined pituitary insufficiency, it is necessary to address each endocrine deficiency. Thyroid stimulating hormone deficiency is often unmasked during the initial phase of rhGH therapy. During the follow-up of GH therapy, every 6 months, FT4 and TSH should be evaluated and, if a decrease is observed, a TRH stimulation test should be performed. Patients with a deficit of ACTH should be placed on the lowest safe maintenance dose of glucocorticoids, no more than 10 mg/m² per day of hydrocortisone, since higher doses may impair the growth response to rhGH therapy. Gonadotropin deficiency may be evident in infancy in a child with microphallus. In patients with CPHD, it is appropriate to begin sex steroid replacement at an appropriate age, since physical and psychological benefits of normalizing sexual maturation must be balanced against the risk of epiphyseal fusion. Assessment of skeletal maturation is useful to prevent rapid epiphyseal closure and loss in adult height. In males, this can be done by beginning at 13–14 years of age with 50 mg testosterone enanthate i.m. every month for about 12 months. Over the next 3–4 years, the dose should gradually be increased to the adult replacement dose of 250 mg every 2–3 weeks. Transdermal testosterone patches and gels have recently become available, which may produce consistent serum levels of testosterone in older adolescents on a stable replacement dose. In girls, therapy involves the use of conjugated oestrogens or ethinyl estradiol: low doses of ethinyl estradiol (20 µg) increased over the next 1–2 years, after which a progestin is added to the last 5–7 days of the cycle to induce bleeding. Once cycling has been induced, it is generally more convenient to use one of the oestrogen-progestin combination oral contraceptive pills [61]. In these patients, fertility is possible with the use of chorionic gonadotropin, human menopausal gonadotropins, GnRH agonist and other fertility medications. Patients also need to understand

that gonadal steroid replacement therapy is necessary for the greater part of their adult life to maintain bone mineral density.

4.1. GH treatment of children after bone marrow transplantation (BMT) for acute leukaemia

Growth impairment and GHD have been frequently observed in children after BMT with TBI and those previously undergoing central nervous system irradiation to treat acute leukaemia [62]. These therapies may compromise growth pubertal development, and consequently final height. However, since the diagnosis of GHD in such patients is made shortly after the end of the irradiation, their growth rate may not be as decreased and their bone age as delayed as in idiopathic GHD children. In our previous paper [63], we showed growth rate impairment both in children who have received TBI and chemotherapy as a preparative regimen and in children receiving prophylactic cranial irradiation before being conditioned with TBI and chemotherapy (**Figure 1**). On the other hand, patients transplanted after a busulfan-containing myeloablative therapy did not experience significant problems in terms of growth velocity [63]. With the increasing number of survivors and duration of follow-up, patients may experience a significant loss of height potential and GH treatment may have beneficial effects. In our GH-treated patients, a successful response was observed with increase in growth rate during the first 2 years of therapy [63]. More recently, other authors found a measurable catch-up growth and a normalization of final height in patients timely treated with GH, thus reducing the

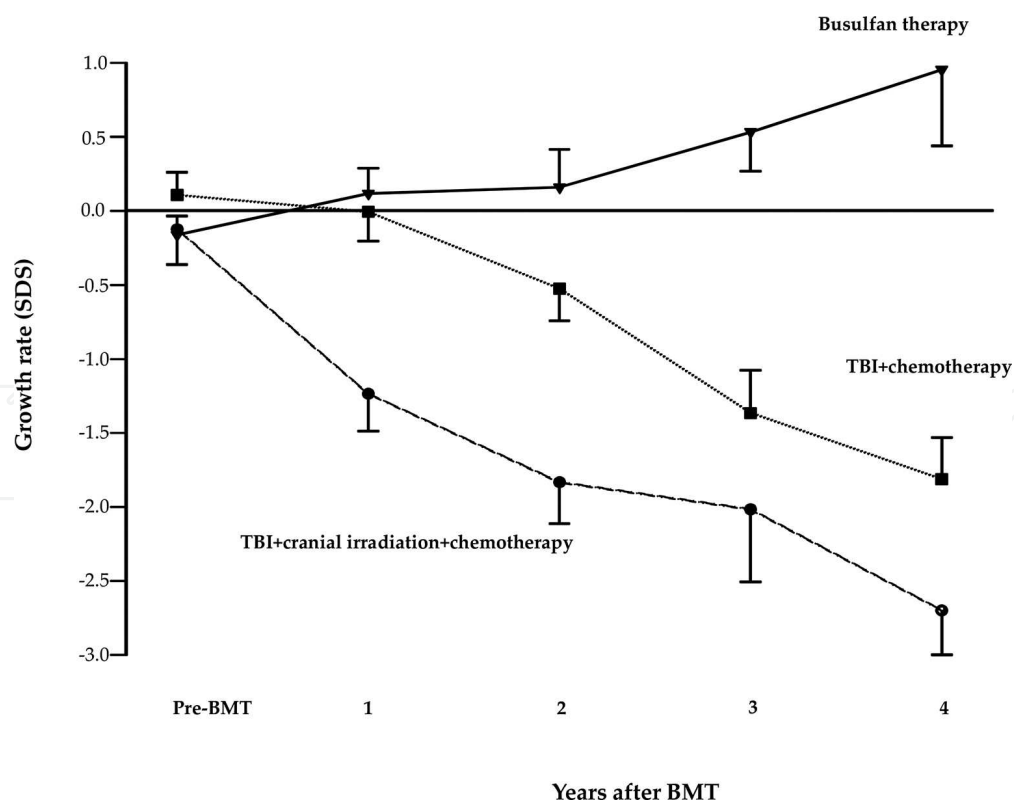


Figure 1. Mean growth rate SDS before and after BMT in the three groups of children with different conditioning regimens before transplantation [63]. Data are expressed as mean \pm standard deviation.

negative impact of the acute leukaemia-related treatment on the growth of paediatric patients [64]. The dosages of GH used in these studies are not different from those used in idiopathic GHD children, ranging from 0.020 to 0.033 mg/kg body weight/day. However, clinicians should be aware that children with prior malignancies show an increase in oncologic risk, especially if treatment of the primary malignancy involved radiation therapy [65].

We therefore propose that all children treated with haematopoietic stem cell transplantation, TBI or central irradiation should routinely undergo, once a year, testing of GH secretion and endocrine evaluation. When GHD is biochemically proven, GH replacement therapy should be considered in order to ameliorate the final growth. However, patients with an active malignancy should not receive GH, and patients whose tumour is no longer active should be carefully monitored for any evidence of progression or recurrence.

4.2. Variability in the response to rhGH therapy

The catch-up growth in response to rhGH therapy is maximal during the first year and typically attenuates during the following years. However, growth velocity is still comparable to healthy age-matched children.

The first-year growth response depends on several factors including dose and frequency of administration and age at the start of rhGH treatment. The first-year growth response is inversely correlated with GH peak during stimulation test and age at the start of replacement therapy and is positively correlated with rhGH dose, weight at the start of replacement therapy and weight at birth. We recently reported that birth size is an important factor affecting the response to GH therapy in GHD children during the first 5 years of treatment. In fact, when GHD children showed small size for their gestational age a blunted response to GH therapy is observed, in comparison to GHD children born with an adequate size and weight for gestational age [66].

Furthermore, the first year growth response to GH may predict up to 7 years of pre-pubertal growth in GHD children and can be used as an aid-in-treatment decision making [67]. Patients with severe GHD show a better growth response in the first year of replacement therapy than those with moderate GHD. For the second, third and fourth years, growth responses are positively correlated with height velocity during the preceding year, weight and weekly rhGH dose, and negatively correlated with chronological age [68]. Recently, the authors of KIGS concluded that IGF-I monitoring during GH therapy is a valuable tool for evaluating compliance and may help clinicians change GH doses to achieve normal serum IGF-I and normal growth [69, 70].

Pharmacogenomic studies have showed that GH receptor (GHR) is the key molecule mediating GH action and that a GHR gene polymorphism (absence of exon 3, d3-GHR) has an influence on the response to rhGH therapy. However, the results of the numerous studies on this issue are still contradictory. Some studies demonstrated that GHD patients carrying the d3-GHR allele had a better response to substitutive GH therapy than patients with the normal allele [71–73], while other studies did not. This may be due to confounding factors such as small sample size, differences in experimental design and severity of GHD [74–78]. In fact, more recent

results from the PREDICT Study demonstrated that response to rhGH depends on the interaction between GHD severity and d3-GHR carriage [79].

Although this d3-GHR genotype was the first identified genetic factor found to modulate the individual response to rhGH therapy, recently, other studies have shown that other polymorphisms of the GHR and of other molecules involved in GH/IGF-I axis (i.e. IGFBP-3 and SOCS2) could have a role in determining the response to rhGH therapy in GHD children [80–82]. These studies may better define the use of GHR polymorphism analysis in clinical practice, moving from pharmacogenetics to routine application and allowing individualization of rhGH doses to optimize final outcome.

4.3. Adherence to GH therapy

Since GH treatment requires regular, daily subcutaneous injections for very long periods, one of the primary causes of GH therapy failure is patient non-adherence to the prescribed drug therapy (daily injections missed or duration of the prescribed regimen not followed), especially in adolescents. No general consensus on the definition of good adherence has been reported and, therefore, the rate of non-adherence to paediatric GH therapy varies between 36% and 49% [83], depending on the detection methods and the definitions used. There is no gold standard method for measuring adherence; the methods most used are direct evaluation of drug levels or its metabolites (urinary GH or serum IGF-I), or indirect evaluation of prescription refills, clinical response, electronic devices or questionnaires completed by patients and/or their parents [84, 85]. Most of these methods are inconvenient for patients or imprecise, underscoring the difficulty for physicians to accurately assess the degree of adherence [86, 87]. The causes of non-adherence are often unknown and may be due to the different lifestyles of the patients, including socio-economic status, level of understanding and type of relationship with the child's physician. Other factors influencing adherence include the complexity of treatment regimens, the long-term nature of the therapy and the discomfort or pain associated with injections [83, 88]. In order to efficaciously improve adherence in GHD patients, appropriate pre-intervention discussion is essential, and should include a clear statement of the short- and long-term treatment targets. Carefully constructed healthcare plans are the key and should include educational programmes, home support and regular reinforcement. Furthermore, it is mandatory to regularly interview patients with a non-aggressive approach to ensure effective communication with patients and their parents [89].

4.4. Side effects of GH therapy

Overall, significant side effects of rhGH therapy in children and adolescents are very rare and include benign intracranial hypertension (frequency between 1/10,000 and 1/1000) and paraesthesia (between 1/1000 and 1/100), arthralgia and myalgia (between 1/100 and 1/10). Recombinant hGH treatment may represent a risk factor for type 2 diabetes in predisposed patients (frequency between 1/10,000 and 1/1000). In general, these events are exaggerated physiologic effects of rhGH (i.e. sodium and water retention, growth rate acceleration) or are due to underlying conditions in treated patients. Management of these side effects may include either a transient reduction of dosage or temporary discontinuation of GH therapy.

In conclusion, the side effects of GH treatment are uncommon in children and the treatment can be considered safe. Only in children with Prader-Willi syndrome treated with GH, have some cases of sudden death been reported due to obstructive sleep apnoea. In any case, these patients deserve particular attention and polysomnography monitoring and otorhinolaryngiatric video endoscopy should be performed before and after beginning GH therapy [90].

The development of growth-inhibiting GH antibodies is extremely rare and limited to the IGHD IA form, although anti-GH antibodies without blocking activity have recently also been detected in a girl with idiopathic GHD [91]. Finally, there is no evidence that rhGH replacement needs to be discontinued during inter-current illness.

Considering the potential for side effects in the use of a growth promoting agent as GH, the community of physicians and pharmaceutical manufacturers have developed systematic methods to survey for short- and long-term effects. Recently, the Safety and Appropriateness of Growth Hormone treatments in Europe (SAGhE) study assembled the largest cohort of GH-treated patients with the longest follow-up for investigating cancer and mortality risks. Interestingly, the analysis was independent of industry. The preliminary results are, however, contrasting [92]. In fact, the French SAGhE has raised concerns of increased mortality risk due to bone tumours or cerebral haemorrhage during follow-up into adulthood, especially in patients who had received high GH doses during childhood [93]. On the contrary, a study on the cohorts from Sweden, Belgium and The Netherlands of the SAGhE reported no increased mortality. The authors showed that the majority of deaths (76%) were caused by accidents or suicides and, more importantly, none of the patients died from cancer or from a cardiovascular disease [94]. More recently, a sponsored observational study (The Hypopituitary Control and Complications Study, HypoCCS) confirmed no increased risk of mortality or incidence of cancer, stroke or myocardial infarction in adult GH-deficient patients who had previously received paediatric GH treatment [95]. These results stress the importance of studies of long-term outcomes after childhood treatments and highlight the need for similar studies to be performed elsewhere.

5. GH therapy in transition age

Growth hormone deficiency may or may not persist into adult life. Patients with childhood onset GHD are usually retested in late adolescence or young adulthood in order to confirm the GHD diagnosis. The 2007 Consensus guidelines for the diagnosis and treatment of GHD adults and its update in 2011 stated that in idiopathic GHD children a re-evaluation by GH stimulation tests is required, unless there is a proven genetic/structural lesion persistent from childhood [96, 97]. At this time, it is important to measure also IGF-I levels and secretion of other pituitary hormones. Re-evaluation of the GH status has shown that GHD is permanent in patients with CPHD, acquired hypothalamic-pituitary lesions, pituitary hypoplasia, pituitary stalk agenesis and posterior pituitary ectopia. On the contrary, a high proportion of children with isolated GHD and no pituitary abnormalities show a different percentage of normalization of GH secretion, ranging from 12.5% to 95% [98–101]. Our recently unpublished

results showed that, at the time of re-testing, 82.1% of severe GHD and 82.4% of partial GHD patients showed transient GHD. This may be due to low reproducibility and high intra-individual variability of the stimulation tests used at the time of diagnosis. Furthermore, there are no clear data on normal GH values after GH stimulation and standardized cut-off levels available in adolescents and young adults. The Consensus guidelines recommended the use of the insulin tolerance test or GHRH+arginine test as provocative tests [97] for re-testing. However, these recommendations are based on limited existing evidence [102, 103] and should be further validated. Growth hormone therapy should be stopped at least 1 month before retesting, although a range of 1–3 months stop has been reported [104].

In the period from late adolescence to early adulthood, GH plays an important role in body composition regulation, muscle mass maturation, full skeletal mineralization and reproductive maturation, as well as in the prevention of metabolic and cardiovascular risk. Therefore, GH replacement should be restarted if a blunted GH secretion is still present [105]. The transition to adult rhGH replacement therapy should be arranged as a close collaboration between the paediatric and adult endocrinologists in order to determine the timing of the patient care transition and to minimize the interruption of GH therapy during the transition period.

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Author details

Mauro Bozzola* and Cristina Meazza

*Address all correspondence to: mauro.bozzola@unipv.it

Internal Medicine and Therapeutics Department, Pediatrics and Adolescentology Unit,
University of Pavia, Pavia, Italy

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