

# **Diagnosis of Growth Hormone Deficiency in Childhood: Progress Report**

**Tommaso Aversa, Filippo De Luca**

**Department of Human Pathology in Adulthood and Childhood “G. Barresi”, University of Messina, Messina, Italy**

## **Abstract**

Growth hormone (GH) deficiency (GHD) is a rare condition with a prevalence of approximately 1 in 4000 during childhood. GHD may be either congenital or acquired. The diagnosis of GHD can be difficult, particularly in idiopathic isolated GHD. There are significant controversies in the diagnosis of GHD in childhood. GH stimulation tests are the key factors in GHD diagnosis, but these tests are poorly reproducible and serum GH concentration measurements can vary significantly according to stimulation tests and GH assays. Close to GH stimulation tests, other biochemical marker, such as IGF-I are useful, together with neuroimaging and genetic tests. These clinical, biochemical and radiological diagnostic tools, including their limits, are discussed in this review.

**KeyWords:** Growth Hormone Deficiency, Growth Hormone stimulation tests, Neuroimaging, Short stature

**IntroducingMember:** Filippo De Luca

**CorrespondingAuthor:** Filippo De Luca, [filippo.deluca@unime.it](mailto:filippo.deluca@unime.it)

## **Introduction**

Growth hormone deficiency (GHD) is a rare condition with a prevalence of approximately 1 in 4000 during childhood (1). However, it is important to make an appropriate and correct diagnosis, as treatment with recombinant human growth hormone (GH) is very effective in improving final height in GHD patients. Conversely, a non-correct diagnosis will lead to unnecessary daily injections, exposure to potential adverse effects and unnecessary costs.

GHD may be either congenital or acquired (Table 1). Congenital causes include genetic mutations (either specific to GH secretion such as GHRHR and GH1 genes, or interfering with pituitary development and multiple pituitary hormone secretion) and structural brain malformations (holoprosencephaly, septo-optic dysplasia, agenesis of corpus callosum, Rathke's cyst). Acquired causes include midline tumors (craniopharyngioma, optic nerve glioma, germinoma, and pituitary adenoma), cranial irradiation, traumatic brain injury, central nervous system infections and inflammatory conditions (sarcoidosis, Langerhan's cell histiocytosis). The diagnosis of GHD can be difficult, particularly in idiopathic isolated GHD (iGHD), i.e. where there is no evidence of multiple pituitary hormone deficiency (MPHD) or visible cranial abnormalities. The efficacy of tools used to diagnose GHD have been widely discussed over the years, due to the lack of a gold

standard diagnostic test. The clinical care pathway that leads to diagnosis includes an assessment of the patient's auxology, a biochemical assessment of the GH-IGF-I axis, and imaging of the hypothalamo-pituitary axis.

Consensus guidelines on the diagnosis of GHD in childhood were published in 2000 by the GH Research Society (2) and the Clinical Care Pathway for GHD of the Italian Society for Pediatric Endocrinology and Diabetes that recently became available on the website (3).

This article reviews the clinical, biochemical and radiological diagnostic tools, including their limits.

## Diagnosis of GHD

In the neonatal period, a random GH measurement of  $<7$  mg/L permits to make a diagnosis of GHD. Outside this period, a single random measurement of serum GH concentrations is of no clinical value as GH secretion is pulsatile with the majority of GH pulses occurring overnight, with very low GH concentrations between pulses. For this reason, provocative tests of GH secretion using physiological/pharmacological stimuli are required to definitely assess GHD diagnosis. GH stimulation tests are based on a priori defined cut-off concentration for GH peak, which allows to distinguish GHD subjects from those who are GH-sufficient.

The lack of a 'gold standard' test for GHD diagnosis has led to the development of arbitrary cut-off levels. Attempts have been made to optimize the cut-off concentration using auxological criteria to define GHD (predominantly height velocity) but these attempts have been hampered, as other disorders can share similar auxology to GHD.

**Table 1.** Etiological classification of Growth Hormone Deficiency (GHD).

Isolated GHD (IGHD)	Genetic <ul style="list-style-type: none"> <li>• GH1 Mutations (GHD type 1A)</li> <li>• GHRH Mutations (GHD type 1B)</li> <li>• GH1 Mutations (GHD type II with evolving pituitary deficiencies)</li> <li>• GHD type III (XL Agammaglobulinemia)</li> <li>• GH1 Kowarski Syndrome (Bioinactive GH)</li> <li>• GHS Mutation/Variant</li> <li>• Alstrom Syndrome</li> </ul>
Multiple Pituitary Hormone Deficiency (MPHD)	Genetic (Transcription factor defect, gene mutation, deletion or duplication) <ul style="list-style-type: none"> <li>• Genes implicated in early development of hypothalamicepituitary region</li> <li>• Genes implicated in early development of brain and hypothalamicepituitary region                             <ul style="list-style-type: none"> <li>- Holoprosencephaly</li> <li>- Septo-optic dysplasia and its spectrum involving eyes</li> <li>- Midline defects (cleft-palate, persistence of craniopharyngeal canal, dental agenesis, ...)</li> <li>- Extra brain malformations (ARNT2, CHD7, IGSF1, ...)</li> <li>- Overlapping Kallmann syndrome (FGF8, FGFR1, PROKR2, PROK2, CDH7, WDR11, ...)</li> <li>- Other conditions</li> </ul> </li> <li>• Genes implicated in cellular differentiation                             <ul style="list-style-type: none"> <li>- Inducing tumor genes (SOX2, BRAF,</li> </ul> </li> </ul>

MPHD	<p>Congenital</p> <ul style="list-style-type: none"> <li>• Midline brain and pituitary developmental defects</li> <li>• Pituitary aplasia; ectopic posterior pituitary, anterior pituitary hypoplasia and pituitary stalk abnormalities (agenesis, hypoplasia); empty sella</li> <li>• Congenital CNS mass (hamartoblastoma, hamartoma), cyst, encephalocele</li> </ul>
IGHD/MPHD	<ul style="list-style-type: none"> <li>• Idiopathic permanent</li> <li>• Idiopathic transitory</li> </ul>
IGHD/MPHD	<p>Acquired</p> <ul style="list-style-type: none"> <li>• CNS tumors (craniopharyngioma, germinoma, ependymoma, pituitary adenoma, meningioma, medulloblastoma, glioma, metastatic tumors (rare), Rathke's cleft cyst, arachnoid cyst)</li> <li>• Radiotherapy (cranial irradiation for CNS tumors, other malignancies, BMT)</li> <li>• TBI (accidental, after neurosurgery, subarachnoid hemorrhage)</li> <li>• Infections (meningitis, encephalitis, tuberculosis, hypophysitis)</li> <li>• Autoimmune (hypophysitis, APS, anti Pit1 antibodies)</li> <li>• Infiltration (LCH, hemochromatosis, chronic blood transfusions)</li> <li>• Chemotherapy (cancer survivors)</li> <li>• GHD, growth hormone deficiency; CPHD, combined pituitary hormone deficiency; MPHD, multiple pituitary hormone deficiency;</li> <li>• CNS, central nervous system; BMT, bone marrow transplantation; TBI, traumatic brain injury; APS, autoimmune polyglandular syndrome; LCH, Langerhans cell histiocytosis.</li> </ul>

When GH stimulation tests were first used in the 1960s, a peak GH concentration after stimulation <5 mg/L was considered mandatory for GHD diagnosis

**GH stimulation tests** (4). During the subsequent years, this cut-off was increased. In Italy, a GH cut-off of 10 mg/L was adopted for a long time, but after 2014 this cut-off has been reduced to 8 mg/L. Recent studies by Wagner et al have suggested a GH peak cut-off of 7 mg/L (5).

The first pharmacological test used to diagnose GHD was the insulin tolerance test. Afterwards several others pharmacological stimuli were identified, including arginine, clonidine, glucagon, levodopa, pyridostigmine, GH releasing hormone (GHRH) and GHRH combined with arginine.

Most of the pharmacological tests are associated with side effects such as nausea, hypotension and somnolence. Very rarely, the insulin tolerance test has been associated with death due to hypoglycemia or its overtreatment with high concentrations of dextrose (6).

Ghigo et al (7) have conducted a study comparing 10 different GH stimulation tests in 472 children without GHD. Mean GH peak concentrations varied between tests from 9.7 mg/L to 61.8 mg/L. Excluding combined stimulation tests, all the tests incorrectly classified some subjects as GH deficient. Using a cut-off of 7 mg/L, false positive rates varied between 8.9% and 23.7%, depending on the test used, and increased to 14.9–49% when the cut-off was increased to 10 mg/L. Variability in GH peak concentration also occurs between tests when used to investigate children with short stature (8). In addition to variability between the GH stimulation tests, the reproducibility of stimulation tests is poor (9).

Current Italian regulations of GH prescription settled by Italian Medicine Agency (AIFA) recommend the use of two pharmacological tests for the diagnosis of GHD, with both tests with a response of less than 8 mg/L. This strategy is used to improve diagnostic accuracy given the large number of false positive results from single stimulation tests in normal children.

### ***Priming for GH stimulation tests***

During puberty, the activation of the hypothalamo-pituitary-gonadal axis leads to a large increase in the circulating concentration of sex steroids with consequent increase of the pulse amplitude of GH secretion, IGF-I concentrations and anterior pituitary size. Children in the peripubertal period and those with delayed puberty often exhibit a growth velocity deceleration and short stature requiring an endocrine assessment. GH testing in this group frequently shows subnormal results. However, these patients often normalize their GH secretion during follow-up as demonstrated by a reassessment of GH secretion during puberty (10). These data led to the suggestion that the primary dysfunction in these patients was sex steroid deficiency, with a probable diagnosis of constitutional delay. The use of estrogen or testosterone to prime the GH axis prior to pharmacological stimulation tests has been demonstrated to increase GH peak concentrations and reduce false positive rates in healthy prepubertal subjects from 39% to 5% (11). There is evidence from one follow-up study of 50 patients that not treating children with subnormal unprimed GH stimulation tests but normal primed GH stimulation tests does not result in an impaired final height (12).

There are three strategies applied to priming by pediatric endocrinologists: a) no priming, b) sex steroid priming for children with pubertal delay (prepubertal at 13–14 years in boys and 11–12 years in girls), c) Sex steroid priming for all prepubertal children (boys >9 years, girls >8 years, this can be based either on chronological age or bone age). Internationally around a third to half of pediatric endocrinologists routinely prime peripubertal children prior to GH stimulation testing.

Common protocols for priming include intramuscular injection of testosterone (100 mg intramuscular, 7–10 days) before testing for boys and the administration of oral estrogen (eg, 10–20 mg

ethinylestradiol) for 48–72 h prior to testing in girls.

### ***Obesity***

Italian prevalence data indicate that 9% of children aged 6-10 years are classified as obese and 21% as overweight. Extensive data in the adult population indicated that spontaneous and stimulated GH secretion is reduced in obesity and has led to the development of different cut-off levels for the diagnosis of GHD in obese or overweight adults (13). Likewise it is clear in childhood that obesity is linked to reduced spontaneous GH secretion (14), reduced peak GH concentrations to stimulation testing and increased rates of diagnosis of GHD compared with lean subjects of similar stature and IGF-I concentrations (15).

A proportion of this increase in GHD diagnosis in obese subjects is likely to be the result of false positive tests due to their obesity, and there is therefore a need for BMI specific cut-off levels for peak GH concentrations during pharmacological stimulation testing in childhood and puberty.

### ***Measurement of IGF-I and IGFBP-3***

Serum IGF-I is mainly derived from the liver under the control of GH and circulates bound to the IGF binding proteins (IGFBPs). There are six classical IGFBPs of which IGFBP-3 is the major serum carrier of IGF-I. Unlike GH, serum concentrations of IGF-I and IGFBP-3 are stable throughout the day.

Serum IGF-I concentrations vary with age and, unfortunately, the normal range for serum IGF-I concentrations in young children overlaps with the range found in children with GHD.

Additionally, IGF-I concentrations are reduced in children with poor nutrition, hypothyroidism, chronic disease, renal failure and diabetes. They also rise dramatically during puberty; thus, in the child with delayed puberty and low growth velocity the IGF-I concentration for age may appear low, although the bone age-adjusted and puberty stage-adjusted IGF-I concentration would be normal.

IGFBP-3 concentrations were thought to be potentially superior to measurement of IGF-I alone as

IGFBP-3 is less nutritionally sensitive than IGF-I. Multiple studies have, however, found no difference in IGFBP-3 concentrations between GHD and non-GHD subjects (16), with a poor sensitivity at 50% and no advantage over measurement of IGF-I alone (17).

### ***Neuroimaging***

The presence of an abnormality within the hypothalamo-pituitary axis provides powerful supporting evidence for a diagnosis of GHD. The most common radiological finding in GHD children is a variable combination of an ectopic posterior pituitary gland, anterior pituitary hypoplasia and a thin or interrupted pituitary stalk (18). Other abnormalities associated with GHD include hypothalamo-pituitary axis tumors such as craniopharyngioma, septo-optic dysplasia, corpus callosum hypoplasia/ agenesis, holoprosencephaly, thickened pituitary stalk (seen in Langerhans cell histiocytosis and germinoma) and the presence of an empty sella.

### ***Genetic investigations***

Mutations in GH1, GHRHR and RNPC3 genes have been identified in patients with isolated GHD, which may be associated with a normal MRI scan (19). The identification of a genetic mutation is particularly useful in supporting the diagnosis in cases of isolated GHD with a normal pituitary MRI. There are many other genes associated with GHD along with other pituitary deficiencies (POU1F1, PROP1, LHX3, LHX4, HESX1, OTX2, SOX2, SOX3, GLI2, GLI3, FGFR1, FGF8 and PROKR2) which are associated with additional clinical and radiological features.

However, in only few patients with congenital MPHD it is possible to detect a causative gene mutation. Therefore, it is fundamental to define some criteria for selecting the patients who should undergo genetic analyses, such as the overall evaluation of hormonal clinical and neuroradiological phenotype (20).

With the increasing clinical availability of genetic technologies such as whole exome and whole genome sequencing, screening for mutations to provide confirmation of the diagnosis of GHD is

likely to increase.

## Conclusions and Recommendations

GHD remains a diagnosis principally based on medical history, clinical features and auxology supported by biochemical and neuroradiological studies.

Problems continue to exist with GH and IGF-I assays for standardization, reproducibility and interassay variability. There are also problems with variability and reproducibility of the pharmacological stimulation tests. The most recent data on the selection of an optimal peak GH concentration come from Wagner et al (5) and could induce the Italian Medicine Agency to further reduce the cut-offs for peak GH levels on the stimulation tests to 7 mg/L.

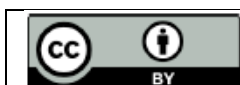
Pituitary MRI is required in all the confirmed patients with GHD.

**Conflicts of Interest:** There is no potential conflict of interest, and the authors have nothing to disclose. This work was not supported by any grant.

## References

1. Murray, P.G., Dattani, M.T., Clayton, P.E. (2016). Controversies in the diagnosis and management of growth hormone deficiency in childhood and adolescence. *Arch Dis Child*. 101(1), 96-100. doi: 10.1136/archdischild-2014-307228.
2. Growth Hormone Research Society (2000). Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. *GH Research Society*. *J ClinEndocrinolMetab*. 85(11), 3990-3993. doi: 10.1210/jcem.85.11.6984.
3. Percorso Diagnostico Terapeutico Assistenziale della Società Italiana di Endocrinologia e Diabetologia Pediatrica (SIEDP/ISPED). Deficit di ormone della crescita in età evolutiva. <http://www.siedp.it/files/PDTAGHD6.5defGV.pdf>
4. Kaplan, S.L., Abrams, C.A., Bell, J.J., Conte, F.A., Grumbach, M.M. (1968). Growth and growth hormone. I. Changes in serum level of growth hormone following hypoglycemia in 134 children with growth retardation. *Pediatr Res*. 2(1), 43-63. doi: 10.1203/00006450-196801000-00005.
5. Wagner, I.V., Paetzold, C., Gausche, R., Vogel, M., Koerner, A., Thiery, J., Arsene, C.G., Henrion, A., Guettler, B., Keller, E., Kiess, W., Pfaeffle, R., Kratzsch, J. (2014). Clinical evidence-based cutoff limits for GH stimulation tests in children with a backup of results with reference to mass spectrometry. *Eur J Endocrinol*. 171(3), 389-397. doi: 10.1530/EJE-14-0165.
6. Shah, A., Stanhope, R., Matthew, D. (1992). Hazards of pharmacological tests of growth hormone secretion in childhood. *BMJ*. 304(6820), 173-174.

7. Ghigo, E., Bellone, J., Aimaretti, G., Bellone, S., Loche, S., Cappa, M., Bartolotta, E., Dammacco, F., Camanni, F. (1996). Reliability of provocative tests to assess growth hormone secretory status. Study in 472 normally growing children. *J ClinEndocrinolMetab.* 81(9), 3323-3327. doi: 10.1210/jcem.81.9.8784091.
8. Secco, A., di Iorgi, N., Napoli, F., Calandra, E., Ghezzi, M., Frassinetti, C., Parodi, S., Casini, M.R., Lorini, R., Loche, S., Maghnie, M. (2009). The glucagon test in the diagnosis of growth hormone deficiency in children with short stature younger than 6 years. *J ClinEndocrinolMetab.* 94(11), 4251-4257. doi: 10.1210/jc.2009-0779.
9. Hilczer, M., Smyczynska, J., Stawerska, R., Lewinski, A. (2006). Stability of IGF-I concentration despite divergent results of repeated GH stimulating tests indicates poor reproducibility of test results. *EndocrRegul.* 40(2), 37-45.
10. Clayton, P.E., Price, D.A., Shalet, S.M. (1987). Growth hormone state after completion of treatment with growth hormone. *Arch Dis Child.* 62(3), 222-226.
11. Marin, G., Domene, H.M., Barnes, K.M., Blackwell, B.J., Cassorla, F.G., Cutler, G.B. Jr. (1994). The effects of estrogen priming and puberty on the growth hormone response to standardized treadmill exercise and arginine-insulin in normal girls and boys. *J ClinEndocrinolMetab.* 79(2), 537-541. doi: 10.1210/jcem.79.2.8045974.
12. Gonc, E.N., Kandemir, N., Ozon, A., Alikasifoglu, A. (2008). Final heights of boys with normal growth hormone responses to provocative tests following priming. *J PediatrEndocrinolMetab.* 21 (10), 963-971.



©2018 by the Author(s); licensee Accademia Peloritana dei Pericolanti (Messina, Italy). This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>).

*Communicated and received June 26, 2018, revised July 23, 2018, published on line December 18, 2018*