DIFFERENTIAL DIAGNOSIS BETWEEN CONSTITUTIONAL DELAY OF GROWTH AND PUBERTY, IDIOPATHIC GROWTH HORMONE DEFICIENCY AND CONGENITAL HYPOGONADOTROPIC HYPOGONADISM: A CLINICAL CHALLENGE FOR THE PEDIATRIC ENDOCRINOLOGIST

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Replay to the reviewers and the Editors

Dear Editor of Minerva Endocrinologica and dear Reviewers, thank you for accepting our paper **“DIFFERENTIAL DIAGNOSIS BETWEEN CONSTITUTIONAL DELAY OF GROWTH AND PUBERTY, IDIOPATHIC GROWTH HORMONE DEFICIENCY AND CONGENITAL HYPOGONADOTROPIC HYPOGONADISM: A CLINICAL CHALLENGE FOR THE PEDIATRIC ENDOCRINOLOGIST”**, code number 3228.

We had performed minor modifications to the paper according to your suggestions, that we are explaining here below, point by point, **in red line**:

Comment 1

General comment (originality, scientific accuracy, strengths and/or weaknesses)

The present review by Galazzi and Persani is a very well written revision of the current literature about the challenging topic of differential diagnosis between cHH, CDGP and IGHD, especially in the peri-pubertal period.

Major corrections (main criticisms)

I have no major corrections

Minor corrections (page, paragraph, line where the author must make the corrections)

I have only the following suggestions:

1. CDGP Endocrine workup, pag 9 line 4: priming is still a controversial issue; I would rather mention not only the Guidelines of 2016 but also the statements about sex priming found in the last ones by Collet-Solberg et al. → **We had added the citation accordingly, thank you**
2. CDGP Genetic tests, pag 13, line 1: authors should better specify what they mean with “shared causes with GHD”. A possible role of GHFR mutation has been reported (Pugliese-Pires PN et al. Eur J Endocrinol. 2011. See also a recent report by the group of Latronico AC in Neuroendocrinology 2019). → **We had specified the phenotype of POU1F1 mutation and added the GHFR mutation in the text and in Figure 1 as well, together with the reference of the paper by Pugliese-Pires PN et al, thank you for these suggestions.**
3. CDGP Final outcomes and therapeutic options, pag 14, line 9: a recent report by Chioma and coll suggests also the use of testosterone gel in CDGP patients (Chioma L et al. Use of testosterone gel compared to intramuscular formulation for puberty induction in males with constitutional delay of growth and puberty: a preliminary study. J Endocrinol Invest. 2018). → **We have added the reference**

by Chioma et al, thank you

4. IGHD Endocrine Workup, pag 16: does it seem that sex steroid priming can be more useful in some tests than others (arginine vs glucagon)? → **Very few papers are actually available in literature exploring the use of a primed vs unprimed growth hormone stimulation tests, especially in the form of randomized controlled trials. The utility of priming before arginine test has been associated with conflicting results in GHD (GH peaks seems to increase in the paper by Molina S, Paoli M, Camacho N, Arata-Bellabarba G, Lanes R. "Is Testosterone and Estrogen Priming Prior to Clonidine Useful in the Evaluation of the Growth Hormone Status of Short Peripubertal Children? "-J Pediatr Endocrinol Metab. 2008 J;21(3) 257-66, but not in the paper written by Martínez AS, Doméné HM, Ropelato MG, Jasper HG, Pennisi PA, Escobar ME, et al. "Estrogen Priming Effect on Growth Hormone (GH) Provocative Test: A Useful Tool for the Diagnosis of GH Deficiency." J Clin Endocrinol Metab. 2000;85(11):4168–72). Indeed, to our knowledge, there are no papers investigating the benefits of primed vs unprimed glucagone tests.**

5. IGHD Endocrine Workup, pag 17, line 4: the latest Guidelines by Collett-Solberg et al report: "the majority of delegates suggested that the threshold be revised to 7 ng/mL" though the previous cut-off (already at 10 mcg/dL) has not been revised yet. Also in Italy, it seems that the cut-off of 8 mcg/dL will be confirmed in the forthcoming revision of nota AIFA 39. → **I had added your sentence with these specifications, thank you.**

6. IGHD Endocrine workup, pag 17, line 21: "abnormal auxological clinical examination", please explain better--> **I had better defined the auxological features warrenting endocrine workup for evalutation of GH reserve, thank you.**

Commento 2

General comment (originality, scientific accuracy, strengths and/or weaknesses)

Overall, the authors have complied a very much needed narrative review of the evidence related to constitutional delay of growth and puberty (CDGP), partial growth hormone deficiency (pGHD), and congenital hypogonadotropic hypogonadism (cHH). They have clearly organised the evidence and provided clear figures. My only hesitation is regarding the study design. In fact, it is a narrative review - although the author refers to it as a 'Personal revision'. I would recommend that it be re-positioned as a narrative review.

Major corrections (main criticisms)

This manuscript is a narrative review of the literature on constitutional delay of growth and puberty (CDGP), partial growth hormone deficiency (pGHD), and

congenital hypogonadotropic hypogonadism (cHH). It may also be important to know some of the historical debates in this area. Also what are the consequences of incorrect diagnosis.

→ We had modified the paper as a narrative review, thank you. We had also added a short introductive chapter at the end of the introduction, explaining the increasing interest in the topic over years as well as the consequences of a failed diagnosis of GHD or cHH.

Minor corrections (page, paragraph, line where the author must make the corrections)

Page 2 line 9. the abbreviation capitalization is incorrect. CHH should be cHH

→ Modified accordingly, sorry about that.

Page 15 line 12. The statement regarding the prevalence of GHD is inaccurate. It is estimated at approximately 1:4,000 to 1:10,000. Please provide more detail to the geographical differences in the world.

→ We had modified the prevalence of GHD for tertiary centers, and we also had added a wider estimation of the prevalence around the world, which seems very much influenced by diagnostic criterias.

Page 22 line 25. prevalence of cHH. again please provide more more detail to the geographical differences in the world.

→ We have added a wider estimation of the prevalence around the world, which seems very much influenced by referral bias. Thank you for the suggestion.

Scheda di revisione editoriale

Bibliografia:

Please, modify References following these examples:

JOURNALS:

Liu H, Li J, Du L, Yang M, Yang D, Li J, et al. Short-term effects of core stability training on the balance and ambulation function of individuals with chronic spinal cord injury: a pilot randomized controlled trial. *Minerva Med* 2019;110:216-23.

HOME PAGE:

AMA: helping doctors help patients. Chicago: American Medical Association; ©1995-2007 [Internet]. Available from: <http://www.ama-assn.org/> [cited 2007, Feb 22].

You have mixed both styles, so please adjust References.

→ Done, thank you. Sorry about that.

Tabelle:

Please, add references in the tables. → Done, thank you.

Figure:

Please, mention Figure 3 in the text. → We had mentioned Figure 3 in the evidence synthesis, thank you.

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DIFFERENTIAL DIAGNOSIS BETWEEN CONSTITUTIONAL DELAY OF GROWTH AND PUBERTY, IDIOPATHIC GROWTH HORMONE DEFICIENCY AND CONGENITAL HYPOGONADOTROPIC HYPOGONADISM: A CLINICAL CHALLENGE FOR THE PEDIATRIC ENDOCRINOLOGIST.

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ABSTRACT

INTRODUCTION: Differential diagnosis between constitutional delay of growth and puberty (CDGP), partial growth hormone deficiency (pGHD), and congenital hypogonadotropic hypogonadism (CHH cHH) may be difficult. All these conditions usually present with poor growth in pre- or peri-pubertal age and they may recur within one familial setting, constituting a highly variable, but somehow common, spectrum of pubertal delay.

EVIDENCE ACQUISITION: ~~Personal~~ Narrative review of the most relevant English papers published between 1981 and march 2020 using the following search terms “constitutional delay of growth and puberty”, “central hypogonadism”, “priming”, “growth hormone deficiency”, “pituitary”, “pituitary Magnetic Resonance Imaging”, with a special regard to the latest scientific acquisitions.

EVIDENCE SYNTHESIS: CDGP is by far the most prevalent entity in boys and recurs within families. pGHD is a rare, often idiopathic and transient condition, where hypostaturism presents more severely. Specificity of pGHD diagnosis is increased by priming children before growth hormone stimulation test (GHST); pituitary MRI and genetic analysis are recommended to personalize future follow-up. Diagnosing CHH may be obvious when anosmia and eunuchoid proportions concomitate. However, CHH can either overlap with pGHD in forms of multiple pituitary hormone deficiencies (MPHD) or syndromic conditions either with CDGP in family pedigrees, so endocrine workup and genetic investigations are necessary. The use of growth charts, bone age, predictors of adult height, primed GHST and low dose sex steroids (LDSS) treatment are recommended.

CONCLUSIONS: Only a step-by-step diagnostic process based on appropriate endocrine and genetic markers together with LDSS treatment can help achieving the correct diagnosis and optimizing outcomes.

Key words:

puberty, short stature, central hypogonadism, growth retardation, pituitary

TEXT

Growth hormone (GH) is the most abundant hormone in the adult pituitary gland. Somatotrophs, located predominantly in the lateral wings of the anterior pituitary, represent the most abundant cell population in the pituitary (35-45% of total). GH action is crucial in mediating growth and metabolic functions in human tissues and, for this reason, is finely regulated. Neuropeptides, neurotransmitters and opiates impinge on the hypothalamus modulating the release of either GHRH, which induces GH gene transcription and hormone release, or somatostatin (also called somatotropin release-inhibiting factor, SRIF), which suppresses both basal and GHRH-stimulated GH pulse amplitude and frequency. Stimulating effects have been described from α -adrenergic pathway (norepinephrine, insulin-induced hypoglycemia, clonidine, arginine, and L-dopa, apomorphine), endorphins/enkephalins (released after physical stress and extreme exercise), from slow-wave sleep, orexigenic hormones (ghrelin and synthetic hexapeptides through growth hormone secretagogue receptor), cholinergic and serotonergic neurons, gut neuropeptides (neurotensin, VIP, motilin, cholecystokinin, glucagone), acute glucocorticoid administration and sex steroids. Inhibiting effects have instead been described from β -adrenergic pathway, anorexigenic hormones (leptine), high circulating fat free acids levels, acute hyperglycemia, hypothyroidism, chronic glucocorticoid exposure, and IGF-1, this last one as part of the hypothalamic-pituitary peripheral regulatory feedback system. The integrated interactions between these influences elicit the classical episodic GH peaks right inside a specific 24-h circadian rhythm. (1)

Gonadotropins (LH and FSH) comprise only 10-15% of the anterior pituitary cell population but their role is crucial for gonadal stimulation and reproduction. Hypothalamic GnRH pulse amplitude and frequency determines the physiologic pattern of LH and FSH secretion. Kisspeptin neurons undergo a dynamic process of prenatal-postnatal maturation that enables them to establish connections to GnRH neurons early in development. KNDy neurons located in the arcuate nucleus of the hypothalamus and co-expressing Kisspeptin, neurokinin B and dynorphin represent the GnRH pulse

generator in both sexes, which then in turn stimulate gonadotroph cells in the pituitary, triggering puberty induction and progression. After a first period of action in utero and in the first months of life (the so-called mini-puberty), this axis is switched off during childhood and reawakens itself in the peripubertal period. Many neurotransmitters from the limbic system, the brain stem and from other hypothalamic nuclei stimulate (glutamate, norepinephrine, kisspeptin) or inhibit (GABA, endogenous opioid, dynorphin) the hypothalamus-pituitary-gonadal (HPG) axis. Other influences may derive from glial cells, peripheral steroids milieu and gonadic peripheral production of activin/inhibin. Leptin, a product of peripheral adipose tissue, is also a positive regulator of the hypothalamic-pituitary-gonadal axis, signaling energy availability to the central nervous system. (2)

(3)

Gonadal steroids then reciprocally have a wide influence on GH secretion and action. Testosterone stimulates central GH secretion mainly through its aromatization to estrogen: in fact, anti-estrogen tamoxifen is able to significantly reduce in normal, and in hypogonadal men on testosterone replacement therapy, the 24-h mean serum GH concentrations, the mean GH pulse amplitudes, and the serum IGF-1 levels. (4) As further proof, the administration of a non-aromatizable androgen (dihydrotestosterone) is not able to elicit GH secretion. (5) Testosterone additionally acts peripherally, amplifying GH-mediated secretion of IGF-1, sodium retention, substrate metabolism and protein anabolism. Exogenous estrogens are instead able to stimulate GH secretion when given orally, due to their first-pass hepatic inhibition of IGF-1 production. This effect can be avoided using transdermal routes, at least if physiological doses of estrogens are given. (6)

Instead it is still unresolved whether, in females pituitaries, estrogens have a central direct stimulatory role on somatotrophs, since stimulated GH secretion proved to be similar between women with premature ovarian failure (either assuming or not hormonal replacement therapy) and aged-matched controls with normal ovarian cycles. (7)

Animal models studies proved the importance of the sex steroids impingement on fetal pituitary somato-mammotrophs cells store, which will subsequently determine the GH reserve in childhood and adulthood. (8) (9) Similarly, human studies have also demonstrated that, physiologically, during puberty the activation of the hypothalamic-pituitary-gonadal axis leads to a large increase in the circulating sex steroids concentration, which then enhance the pulse amplitude of GH secretion, increase IGF-I concentrations and the anterior pituitary size. (5) (10)

Many association studies in normal children have also proved a physiological threefold increase in GH secretion following the increase in gonadal steroid concentrations during puberty. (11)

GH secretion has also proved to be reduced in hypogonadal children (12) but increased in precocious puberty, whose successful therapy is able to normalize GH secretion. (13)

Because of these multiple interconnections between GH and HPG axis, it is not infrequent that a child in peripubertal age may present to a pediatric endocrinologist with short stature and/or reduced growth velocity with associated delayed puberty. [Table I]. For the same reasons, subnormal GH peaks to physiological or pharmacological stimuli are frequently found in normal prepubertal children, but they have proven to fully normalize when they will achieve stages 4 or 5 of Tanner maturation or after estrogen administration. (14)

While constitutional delay of growth and puberty (CDGP) may be the most probable diagnosis in a child presenting with short stature and pubertal delay, especially for males, growth hormone deficiency and congenital hypogonadotropic hypogonadism must at the same time be ruled out.

Otherwise, in children with true GHD or cHH left untreated, adult height and pubertal maturation will lack to fully develop normally, resulting in important psycho-social discomfort as well as long term medical sequelae in these children (permanent short stature in GHD, cryptorchidism and risk of testicular cancers/lack of fertility in cHH, osteoporosis and alteration of body composition in both conditions). As growth is a good indicator of a child's health, the increasing attention on children wellness and well-being especially in the more developed countries has been a matter of medical

1 attention over years. Moreover, as the child grows into adulthood, fertility potential becomes an
2
3 important issue, which is also a major concern for parents.
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EVIDENCE ACQUISITION

1. Constitutional delay of growth and puberty (CDGP)

1.A Definition

The term CDGP describes children who have a normal variant of maturation tempo characterized by short stature with relatively normal growth rate during infancy, but showing deceleration during peribubertal period possibly due to the lack of growth spurt physiologically driven by the sex hormones rise. According to the pubertal status, 3 different “categories” of CDGP children may be defined:

- CDGP type 1: Prepubertal children (Tanner stage G1 for boys ≥ 14 yo or B1 for girls ≥ 13 yo), as reported by the 2007 ESPE classification, (15) usually associated by slow growth rate, delayed bone age (delay of 1 year or more compared to chronological age) and a positive family history of delayed puberty
- CDGP type 2: Pubertal children but displaying slow or stuttering progression of sexual characteristics, with a genital Tanner stage less than – 2 SDS compared to normal age-matched peers, defined by the use of puberty nomograms (16)
- Possible CDGP type 3, according to Stalman et al: if before 14 (for boys) or 13 (for girls) years old if, during his/her growth, three out of four conditions are met: i) Tanner stage < -1 SDS defined with the use of puberty nomograms; ii) bone age delay > 1 year compared to chronological age; iii) being short for target height (TH); and iv) positive family history of pubertal delay (menarche in the mother > 15 yo; late paternal growth spurt and shaving). (17)

1.B Prevalence

CDGP is a relative common condition, affecting 2-2.5% of the population. (18) This alteration is extremely common in boys, and less in girls. It is estimated that up to 80% of boys and around 33% of girls displaying delayed puberty have a self-limited condition. (19) However, prevalence in CDGP females ranges widely : in a large Georgian cohort of female adolescents with delayed puberty, for example, the prevalence of CDGP in girls is accounted to be lower and around 10%, against the great majority of the female cohort displaying organic diseases (premature ovarian failure/absence of uterus or vagina). (20) Because in family series reports males and females are described to be equally affected (19), a gender selection bias seeking medical attention has been postulated. (21)

1.C Clinical presentation

Children displaying CDGP may frequently seek for medical attention for short stature, which is usually not severe (> -2.5 SDS) and for reduced growth velocity (< -2 SDS) together with a prepubertal stage of sex maturation. Although growth velocity (GV) may be reduced compared to normal pubertal children, it is frequently found to be > 3.6 cm/year in boys. On the other hand, GV seems not discriminative at best for different etiologies of delayed puberty in girls. (22)

Because CDGP patients are sometimes poor eaters (23) and need higher caloric intake compared to age-matched controls (24), BMI is often found to be at lower range, which is an important concern for parents and contributes possibly independently to the reduced growth. Of interest, nearly 25% of boys with CDGP come with the diagnosis of attention deficit with hyperactivity disorder (24). Even though adrenarche/pubarche derive from adrenal rather than HPG axis activation, delayed puberty in these patients consists of a delayed gonadarche together with absence of pubarche/adrenarche. (25)

Looking at growth charts may be useful. CDGP boys are born with normal length, have a decreased growth pattern compared to target percentile until 2 years old, then tend to level till 5 years old,

starting from when a height SDS loss is already detectable, reaching its maximum at pubertal age.
(26)

For a complete auxological workup, a systematic assessment of bone age is warranted, either using Greulich-Pyle or Tanner Whitehouse methods (referring to its second edition of 1983 TW2, or its third revised edition of 2001 TW3) or informatic programs that are rising into attention for the more accurate bone age assessment in non-american non-caucasian ethnicities. (27) In CDGP subjects bone age is frequently found to be delayed by at least 2 years compared to chronological age, even though it can range from 1 to 3 years less, so that if we bring the child back on his biological age the growth percentile may be adequate for the family target. (28)

The prediction of adult height based on bone age ($PAH =$ predicted adult height) may also be very useful because it frequently suggest that a child with CDGP will lay within the lower range of his/her target height (29). To this regard it is worth explaining that different methods for final height prediction based on bone age exist, and according to the method used there may be some overestimations or undestimations of the predicted final height. (**Table II**) In the matter of question, the most used Bayley and Pinneaud system has been proved to overestimate boy's final height, raising doubts of the possibility of truly rely on this method for adult height prediction. (29) For this reasons, new models of bone age prediction in CDGP patients have been recently established. (30)

Clinical history and examination of the child should point out the so called "red flags" i.e. cues orienting the clinician towards specific diagnostic subgroups (**Fig. 2**). To this extent it is worth noticing that in CDGP patients cryptorchidism may be found in a low percentage of cases (2%) mostly as a unilateral finding. (18) Up to 80% of children diagnosed as CDGPs have at least one family member in which self-limited delayed puberty has occurred, which can be also taken as an important diagnostic tool; however, a family history of CDGP does not exclude cHH given the incomplete penetrance and variable expressivity of cHH phenotype within pedigrees (of which delayed puberty,

subfertility and hypo-anosmia can also be enriched). (2) (3) A new observation of our group introduced into this complex clinical picture also TBX3 mutations accounting for Ulnar Mammary Syndrome (UMS), and several reports of UMS families describe the variable association of either CDGP, more frequently, either of true cHH defects and GHD, in a minority of cases (31). **(Figure 2)**

1.D Endocrine workup and the role of sex steroid priming before growth hormone stimulation tests

Priming refers to the administration of sex steroids to prepubertal or early pubertal children prior to GHST. The evidence to support this practice is low, however suggested by the 2016 International Guidelines for growth hormone deficiency and idiopathic short stature. According to these guidelines, priming is suggested for prepubertal boys older than 11 or prepubertal girls older than 10 years with adult height prognosis within -2 SD of the reference population mean in order to prevent unnecessary rhGH treatment of children with a more probable diagnosis of constitutional delay of growth and puberty. (32) (33)

The studies supporting this approach are however of limited evidence, considering the small group of patients of the studies analyzed, yet sometimes conflicting. Marin et al showed a 95% rate of normalization in 11 normal children after administration of ethinylestradiol (EE) $40 \mu\text{g}/\text{m}^2$ in 3 divided doses with meals for 2 days and, compared to placebo, a rise in both GH peak from 7.6 ± 4.4 on placebo to $18.7 \pm 9.2 \mu\text{g}/\text{L}$ on EE and of the lower limit of the 95° confidence interval from 1.7 to $7.2 \mu\text{g}/\text{L}$. (14) Martinez et al, similarly, showed in a randomized control study a significant increase in GH peaks under a sequential arginine-clonidine test when 44 short normal children were primed with estradiol valerate (E2) orally (1 or 2 mg depending on the weight $<$ or $>$ of 20 kg, respectively) in three consecutive days before testing, compared to placebo: in these children GH mean peak

increased from 17.8 ± 10.9 on placebo to 27.9 ± 14.5 $\mu\text{g/L}$ on E2, increasing diagnostic accuracy of GHST from 90% to 95%. (34) Analogously, Molina et al proved that 39 children primed with either 100 mg i.m. of testosterone enanthate for 5-8 days (in boys) or with 1 mg estradiol valerate daily for 3 days (in girls) were able to significantly increase GH mean peaks under clonidine stimulation test, either if diagnosed as CDGP (from 5.83 ± 2.67 to 17.42 ± 8.46 $\mu\text{g/L}$) or as GHD (from 3.68 ± 2.34 to 5.95 ± 2.76 $\mu\text{g/L}$). (35) Muller et al showed a normalization (GH peak > 10 $\mu\text{g/L}$) after arginine test in 77% of 26 peripubertal boys primed with 100 mg of testosterone enanthate 3-10 days before the stimulation. (36) Taken together, these studies led to the suggestion that estrogen priming may reduce the number of false positive children (i.e. false non responders to a GHST) avoiding unnecessary rhGH treatment in a high proportion of children under assessment for short stature.

Other studies have however shown opposite results. Soliman et al proved that in children above 9 years of age under investigation for short stature with a clonidine stimulation test, the proportion of patients displaying GH peaks > 10 $\mu\text{g/L}$ did not differ between the primed group (pre-treated with 25 mg of intramuscular testosterone depot 7-10 days before the test for boys or with 1,25 mg of oral conjugated estrogens for 3 days before the test for girls) compared to the unprimed group (52% vs 47% respectively, N.S). (37) Most importantly, only one follow-up study on 50 boys was able to prove that avoiding treatment in children with subnormal unprimed but normal primed GH peaks under GHST does not result in an impaired final height compared to their midparental target (-1.27 vs -1.38 SDS, respectively). (38)

Larger follow-up studies considering both males and females and looking at final height of children undergoing primed GHST are lacking. However, taken together, these studies evoke the idea that priming before GHST may reduce the false positive diagnosis of growth hormone deficiency, avoiding unnecessary rhGH treatment in many children under investigation for short stature. It is however estimated that only 30-40% of pediatric endocrinologists prime peripubertal children prior to GH stimulation testing in routine clinical practice. The limited diagnostic application is justified

by some experts based on considerations of priming as an artificial stimulus generating false transient normal GH peaks under GHST. (39)

Beyond GHST, no endocrine marker can be fully reliable in the diagnosis of CDGP. Nevertheless, in males, measurable levels of Inhibin B (> 35 ng/mL) have been proposed to fairly correlate with the maturation of Sertoli cells in the gonads, hence indirectly proving that, in the first months of life, mini-puberty has occurred, pointing out a CDGP rather than cHH diagnosis. (40) AMH has also proved to be slightly higher in prepubertal CDGP compared to cHH, however not discriminative for peripubertal CDGP (Tanner stage 2) where it is found to be lower, thus not giving advantages compared to inhibin B alone. (40) (41) In prepubertal CDGP the combination of Inhibin B at a cut-off ≥ 28.5 ng/L with AMH at a cut-off of ≥ 20 ng/mL has however proved to increase the specificity of CDGP diagnosis up to 83%. (41) Other markers of testicular (INSL3), and adrenal (DHEAS) activity have not proved to be discriminative for the differential diagnosis between CDGP and cHH. (41)

The restriction of most of the studies to the male cohorts does not allow to use these endocrine markers for girls. At the same time, in females Inhibin B levels are less useful as they are lower and a less significant rise is seen at puberty. (42)

Basal gonadotropin levels are not discriminative as they are supposed to be low either in prepubertal CDGP or in cHH cohorts. If in CDGP children puberty is in a very initial stage, basal gonadotropins levels may be found to be more than 0.2 UI/L in both genders with the use of immunochemiluminometric assays, which could be a simple and useful marker against a severe cHH form. (43) Moreover, HPG axis stimulation upon GnRH test or following a GnRH agonist (GnRHa) has also been proposed (22) (42) (43), however up to 30% of children diagnosed as either CDGP or cHH overlap their LH peaks under stimulation. (44) Despite some promises for longer GnRH test protocols (up to 36 hours), these tests have suggested to be helpful only in a few studies but without

high diagnostic accuracy. Similarly, the use of GnRHa (Naferelin, Leuprolide, Triptorelin, Buserelin) has proved to elicit higher LH peaks but the low sample size of these studies, the presence of overlapping results with cHH cohorts and the absence of a clear diagnostic threshold limit their diagnostic utility. (44)

HCG test has also been postulated as a useful diagnostic tool for the differential diagnosis between CDGP and cHH (see below section 3.D). (45) (46).

Kisspeptine boluses have also proved to heterogeneously increase LH responses in CDGP patients (47) in research settings, but this approach is not yet routinely performed in clinical practice.

Another important tool that can be taken into account to differentiate CDGP from other cohorts is the evidence of a self progressing pubertal development after a 3-6 months treatment trial with low dose sex steroids (LDSS). (3) (48) After LDSS withdrawal, an Inhibin B > 94.7 ng/mL and LH peak after GnRHa > 14.7 U/L have been also proven to discriminate at best CDGP upon cHH. (49)

1.E GENETIC TESTS

Genetic basis of CDGP are supposed to be different from those of cHH. Even though, heterozygous variants in cHH putative genes have been found in up to 7% of the CDGP cohorts (18), the current scientific literature tends to consider the hereditary basis of CDGP as a different, yet mainly still unknown, genetic field. Observational studies have demonstrated that several different inheritance patterns can occur in CDGP, including autosomal dominant pattern (in the majority of the cases, with or without complete penetrance), autosomal recessive, bilinear (both parents affected) or X-linked. De novo mutations can also occur. (19)

Four genes clusters have been demonstrated in CDGP cohorts by Howard et al. with exome sequencing in Finnish CDGP index patients: (3)

- 1) Fat Mass and Obesity-associated (FTO) gene (in three of 67 families)
- 2) Heparan Sulfate 6-O-Sulfotransferase 1 (HS6ST1) gene (in one in 67 families)
- 3) Enhanced At Puberty 1 (EAP1) gene (in two in 67 families)
- 4) IGSF10 rare sequence variants in up to 16.4% of the CDGP cohorts, a gene which encodes a protein that belongs to the immunoglobulin superfamily. However, the role of IGFS10 in delayed puberty remains open, as in a follow-up study, IGSF10 mutations were not reported to be enriched in CDGP patients when compared to controls.

Mutations in the pituitary trascription factor POU1F1 (PIT1) involved in pituitary thyrotrophs and lacto-somatotrophs cells development and hormonal expression may present with pubrrtal delay or with absent or delayed adrenarche, resembling delayed puberty (50). Mutations in GHSR, a growth hormone secretagogue receptor gene whose endogenous ligand ghrelin produced from gastric cells increases GH pituitary secretion, have been found to be mutated in both GHD and CDGP patients.

(51)

Some known syndromic conditions may present with either CDGP, GHD or cHH within family pedigrees (Prader Willy Syndrome or CHARGE syndrome). Moreover, as already mentioned, TBX3 mutations accounting for Ulnar Mammary Syndrome (UMS) have been described in litterature also linked to all of these scenarios including CDGP in many family pedigrees. (31).

The genetic basis of CDGP and shared causes with GHD and cHH are illustrated in **Figure 1**.

1.F FINAL OUTCOMES AND THERAPEUTIC OPTIONS

Given that CDGP is a self-limiting condition, reassurance and watchful waiting are reasonable approaches to treatment. On the other hand, however, new evidences towards long-term sequelae

associated with delayed puberty are rising into clinician's attention, from the possibility of early menopause and low bone density to a worse psychosocial well-being and peer's relationships (19). Moreover, association studies investigating earlier or later timing in puberty in both genders have been linked with a wide range of adverse health outcomes, including cancers, cardiometabolic, gastrointestinal, musculoskeletal and neurological pathologies. (52)

Additionally, because in CDGPs the magnitude of the growth spurt may be blunted as the child gets older, not infrequently these children will not be able to reach their full growth potential. (53) To this regard, final height in CDGP patients has been widely investigated in boys and many studies have proven that untreated CDGP patients are prone to become shorter either compared to their mid parental targets and/or their peers displaying normal timing of puberty, whereas if a treatment with LDSS is given to the child for a short period a time, FH has proved to become similar or better compared to those left untreated. (54) (55) (56) (57). (**Table III**) This effect may expire if higher doses of testosterone are used. (58) Testosterone therapy (in the form of testosterone esters/undecanoate, given either intramuscularly, transdermal gel, or orally) in this group of patients appears a safe and well tolerated approach that can favor a prompt recovery of hypothalamus-pituitary axis after discontinuation of treatment if low doses are given. (59) The treatment schedules are quite variable, and the efficacy of the treatment is clearly an under-investigated area in adolescent medicine with very few randomised trials available. Letrozole treatment has also been proven to give benefits in CDGP boys compared to testosterone in terms of pubertal progression, even by eliciting different HPG axis responses (increasing gonadotropins and Inhibin B, leading to larger testis size). (22) Both therapies (testosterone 1mg/kg/month or letrozole 2.5 mg/day) had anyway proven to elicit changes in Sertoli cells markers (i.e. decrease in AMH), reassuring about safety in developing mature seminiferous epithelium with both approaches. (60) Other therapies such as oxandrolone have also been suggested for treatment of CDGP, but long term data are lacking. (61) (62)

Very few follow-up studies are instead available for CDGP girls, and data on treatment with low dose estradiol are almost lacking. (57) (63) (**Table III**) This is possibly due to the lower rate of presentation to medical attention of girls with pubertal delay compared to boys (26). For girls, natural and transdermal estradiol preparations are preferred.

A reasonable therapeutic approach for CDGP children may be made either with 50 mg testosterone enanthate intramuscularly monthly for 3-6 months either with 10 mg testosterone gel 2% daily for 3 months for boys, or with ¼ of a 25 µg patch of 17-β estradiol once or twice weekly for 6 months for girls. (3) (64) (65)

Taken together we believe that CDGPs should not be considered as a para-physiological variant of maturation-tempo but, perhaps more appropriately, as a condition characterized by a significant delay of pubertal timing needing treatment with LDSS for a short period of time in order to normalize their growth pattern and achieve full growth potential and limit psychosocial and medical issues associated with delayed puberty.

2. Idiopathic growth hormone deficiency

2.A DEFINITION:

Growth hormone deficiency is a complex yet rare diagnosis in a child, that should be based on the whole thing of auxological, anatomic and laboratory's findings. (33) This condition can be challenging to differentiate from CDGP as in both settings there is a height SDS deflection and relatively low height velocity compared with cross-sectional population references. Moreover, GHD may also overlap with cHH clinically, especially when multiple pituitary hormone deficiencies (MPHD) or shared genetic mutations with the condition occurs.

2.B PREVALENCE

The prevalence of GHD is around 1 child every 4.000-10.000. It is therefore a rare condition and mainly a diagnosis of exclusion upon other possible causes of poor growth (malnutrition, chronic liver and renal diseases, pulmonary diseases, syndromic conditions, iron overload, hypothyroidism, diabetes, skeletal dysplasias, inborn errors of metabolism, and CDGP).

Prevalence of GHD worldwide may range widely due to the lack of standard diagnostic criteria (for example, it has been overestimated as up to 1:1800 in Sri Lanka probably due to more liberal diagnostic standards) and also due to referral bias. Even though there are no apparent racial differences in the incidence of GHD, The National Cooperative Growth Study (NCGS), Genentech's study on North American database, revealed that 85% of patients receiving GH treatment for idiopathic GHD were white, 6% were black, and 2% were Asian and similar distributions were seen with patients with other forms of short stature. Additionally, patients with GHD from organic causes such as tumors and radiation, may be much more prevalent in more industrialized countries, where medical intervention on population is deepened. (66).

2.C CLINICAL PRESENTATION

Children with GHD may display severe harmonic short stature, a reduced height compared to the target height (TH) and/or a poor growth rate. The past medical history may help the clinician if neonatal hypoglycemia, midline defects, prolonged jaundice or MPHDs are present. (67)

In growth charts of boys with congenital GHD a continuous decrease in height SDS from birth on occurs without any levelling (68). Moreover, an increase in fat mass has been described in patients left untreated giving them a "cherubic appearance", which can also be taken as an important diagnostic tool against a presumptive CDGP diagnosis, where BMI is classically found to be low.

(30)

Bone age, either assessed with Greulich & Pyle or Tanner-Whitehouse methods, is again found to be delayed due to the low GH-IGF-1 levels modelling bone maturation. However, there may be three possible confounding scenarios in which bone age may not be delayed: (i) recently acquired GHD, (ii) obesity, which is typically associated with advanced bone age, and (iii) concomitant precocious puberty, a scenario where sex hormones can trigger bone maturation. (33)

PAH based on bone age will clearly show an adult height well below the genetic potential of the child. As already mentioned, many methods for PAH predictions exist (**Table II**): target height, which is the most used method of height prediction in clinical practice, has anyway been associated with a higher prediction error compared to the less used Roche-Weiner-Thissen method (RWT) in a cohort of severe GHD. (69) Therefore, this method might be superior for the prediction of adult height in a child with short stature. It is however clear that CDGP can come into differential diagnosis mainly with cases of recently acquired GHD or, more frequently, partial idiopathic GHD (pGHD).

2.D ENDOCRINE WORKUP, role of sex steroid priming before GHST and pituitary MRI

Serum IGF-1 levels are frequently low in GHD compared to pubertal aged-matched peers and its determination in peripubertal children should be assessed within adjusted reference ranges for actual pubertal status. (70) An IGF-1 level > 0 SDS for gender and age specific range makes the GHD diagnosis highly unlikely (71) and it can be used as a screening tool for GHD. However, when IGF-1 is found to be low, a series of other conditions need to be excluded (such as malnutrition, chronic diseases, hepatic diseases).

However, IGF-1 levels may be influenced by a series of other conditions, therefore an evaluation of GH reserve in the majority of cases is warranted.

Regarding growth hormone stimulation tests, in the absence of a “gold standard”, it is impossible to precisely define the sensitivity and the specificity of any test for GHD. Insulin tolerance test (ITT) may be considered the gold standard for the evaluation of GH reserve, but its intrinsic risks hamper the definition of normal responses thresholds. Some information in specificity can be learned by comparing the results obtained in normal children. It is well known that within normal children and/or children with idiopathic short stature 10 to 25% will show a GH peak $< 7 \mu\text{g/L}$ and up to 23-49% will have a peak $< 10 \mu\text{g/L}$. (72) This may suggest that every individual stimulus may incorrectly classify almost half of the children as false positive GHD, if higher cut-offs are assumed. To this regard, the definition of normal GH peak under stimuli has been changed and progressively reduced over years and a further reduction of the diagnostic cut-off of GH peak by $7 \mu\text{g/L}$ has been recently proposed by the latest Growth Hormone Research Society Guidelines the definition of normal GH peak under stimuli an attempt of progressively reducing the diagnostic cut-off of GH peak by $7 \mu\text{g/L}$ has been recently proposed by the latest Growth Hormone Research Society Guidelines (33), although the previous cut-off (at 10 mcg/dL) has not been revised yet. Also in Italy, the cut-off of 8 mcg/dL has just been confirmed in the latest revision of nota AIFA 39.

Sensitivity of GHST, can hence be deduced by comparing one positive test to the positive response of another investigation, for example correlating low IGF-1 concentrations to the failed results on GHST. However sometimes the tests can be discrepant and in this scenario it is difficult to determine whether this is due to the poor sensitivity of the IGF-1 test or to the poor specificity of the GHSTs themselves.

To increase GHST specificity, sex steroids priming preparation should be performed before testing. However, in contrast with CDGP cohorts, sex steroid priming before growth hormone stimulation testing has been associated with conflicting benefits in the GHD cohorts in literature. Even though in the already cited study of Molina primed GHD children were able to significantly increase GH mean peaks under clonidine stimulation test from 3.68 ± 2.34 to $5.95 \pm 2.76 \mu\text{g/L}$. (35), in the study

performed by Martinez et al, GHD children did not significantly increase GH mean peaks under sequential arginine-clonidine test after estrogen priming (GH peak 3.1 ± 2.4 on placebo and 4.5 ± 2.7 $\mu\text{g/L}$ on E2, NS), suggesting that the effect of priming is irrelevant in those patients with more severe forms of GHD, thus displaying lower GH peaks under stimuli. (34)

Because of the poor specificity and the low reproducibility of the GHST, the cardinal tool for a correct diagnosis is again ~~an abnormal auxological-clinical examination, considered to be the gold standard.~~ a pathological short stature and/or growth deceleration (i.e. height < -2 SDS, difference from target height < -1.5 SDS, growth velocity < -2 SDS) highlighted by clinical examination, considered to be the undeniable starting point. Pathological auxological criterias are in fact thought to increase the pre-test probability of GHD in children under investigation, especially in those without any other risk factor (i.e. pathology including ectopic posterior pituitary and/or abnormality of the pituitary stalk, irradiation, at least other pituitary hormone deficiency or carrying a pathogenic genetic mutation), and in these patients two abnormal tests are frequently needed before GHD is diagnosed. (32) Instead, in a patient with pathologic auxological criteria, a normal GH peak in one test rules out GHD in most of the cases. However, as in some conditions (cranial irradiation, optic nerve hypoplasia, traumatic brain injury, or known genetic conditions) GHD may evolve over time, a second GHST is frequently suggested at a later time point. (33)

A Magnetic Resonance Imaging (MRI) of the hypothalamus and pituitary glands should be performed in all patients diagnosed with GHD in order to rule out anatomical defects of the hypothalamic-pituitary region or tumoral brain lesions. This is important for predicting the likelihood of other pituitary deficiencies, the utility of genetic testing and the probability of persistent GHD after attainment of final height. (33) In around 15% of cases an anatomic malformation of the hypothalamus-pituitary axis is found, but in the majority of the children, no cause can be identified and this group of patients are often referred as having idiopathic growth hormone deficiency (IGHD). (73) These children diagnosed as IGHD frequently carry transient forms of growth hormone

deficiency during childhood and the rate of normal retesting has been found to be as high as 80% in many papers (see later). (74) (75) (76) (77)

Major anatomical abnormalities (including thin or interrupted hypophyseal stalk, suprasellar or intrasellar tumors invading the hypothalamus-pituitary anatomy) are known risk factors for multiple pituitary hormonal deficiencies (MPHDs) and of persistent GHD in adulthood. (74) (75) (78) Ectopic posterior pituitary with an otherwise normal anterior pituitary gland is also considered to increase by 26% the probability of adult persistent GHD (79). A small anterior pituitary, defined as a gland < -2 SDS of normal size using the few available standards for normal population categorized for age and gender (80) (81) (82), may incidentally be found on MRI. A hypoplastic anterior pituitary gland by itself is not sufficient to diagnose GHD, but may indicate the need for a more extensive evaluation of the anterior pituitary function. (33) Pituitary size should be interpreted in the context of pubertal status. It is in fact well established in normal subjects that the pituitary gland increases its size during puberty (a phenomenon even more pronounced in females), reaching a plateau at approximately 20 years old, and a physiologic decline thereafter. (83)

Many Authors studying pituitary MRI of IGHD children have indeed found a significantly low volume of the pituitary gland compared to age-matched peers, speculating that there could be an association between anatomic and functional abnormalities, meaning that those patients with smaller pituitary glands may have a smaller somatotrophs volume thus secreting less growth hormone. (84) (85)

2.E GENETIC TESTS

The majority of patients with growth hormone deficiency is considered of idiopathic origin. Some isolated growth hormone deficiency are secondary to a genetic mutation within the GH gene locus named GH1 (IGHD type IA or IB) or in its introns (IGHD type II), or may be transmitted as an X-linked trait together with hypogammaglobulinemia (IGHD type III) (73).

An exome or a genome wide approach is nowadays thought to be the best diagnostic option, yet sometimes not available in clinical settings. A panel-oriented gene analysis for the most frequent genes associated with GHD could be a reasonable approach. Some features are associated with a high pre-test probability of a genetic mutation: severe short stature at presentation (< -3 SDS from population or from target height), multiple pituitary hormonal deficiencies, anatomic alteration of the CNS, familial segregation with an autosomal dominant or recessive pattern or with a history of consanguinity. (33) Genes associated with GHD and shared with CDGP and cHH are illustrated in **Figure 2**.

2.F FINAL OUTCOMES AND THERAPEUTIC OPTIONS

Even though growth hormone therapy has been given since 1958, starting with extracted GH from human pituitary glands, and then from 1980s with the use of the recombinant human GH (rhGH) technology, little is still known about its effect on adult height and conflicting data exist on long-term studies, with some showing failure to reach the genetic mid-parental target height but others, by contrary, showing successful target height achievements. It is clear from reports that individual height response may vary considerably even with individualized treatment regimens, mainly depending on the chronological age at the start of therapy, on the severity of the GH deficiency after stimulation tests and on the stage of pubertal development. (86) (87) (88) (89) (90) (**Table IV**).

In fact, as a result of the lack of a uniformed cut-off limit for GHD, of the heterogeneity of the GHST used, and the fact that sex steroid priming is frequently not performed, it is not infrequent that a lot of these studies on IGHD children erroneously include also CDGP patients, who may present with short stature and pubertal delay. For this reason, outcomes reported in papers on final height of patients receiving rhGH treatment may vary widely and in some cases an overlap in terms of FH outcomes is seen with CDGP cohorts, especially for CDGP girls or for CDGP boys treated with

LDSS. (Tables III and IV). If very low growth outcomes are achieved after treatment with rhGH (SDS FH-IH around +1.2 only), possible other causes of short stature should be considered. (91)

Roughly, a standard substitutive dose of rhGH should be 25-35 $\mu\text{g/kg/die}$ but the dose of rhGH should then be individualized according to GH responsiveness aiming for the lowest dose able to induce a response in height velocity, possibly using prediction models. (92)

Retesting GH status at end of growth is currently recommended by the European Society for Pediatric Endocrinology guidelines for all patients with GHD except for those children with multiple (≥ 3) pituitary hormone deficiencies regardless of the etiology, for GHD with a documented causal genetic mutation or specific pituitary/ hypothalamic structural defect. (32) To this extent, small pituitary glands are not considered as anatomic defects, since a high proportion of normalization at retesting in children with this condition has been demonstrated (74)

Guidelines differ on suggested cut-offs for GHD diagnosis during transition under ITT test, with American guidelines using lower cut-offs (5 $\mu\text{g/L}$) (93), European Society for Pediatric Endocrinology guidelines suggesting a cut-off of 5.6 $\mu\text{g/L}$ (94) for immunometric assays, and GH Research Society guidelines suggesting a cut-off of 6 mg/L . (33)

Studies using standard cut-offs have confirmed that around two thirds of patients diagnosed as GHD during childhood-puberty, proved to have transient form of GHD. Tauber et al reported a 67% rate of normalization at retesting in IGHD patients, which was increased up to 71% in children previously diagnosed as partial GHD (GH peak at diagnosis between 5-10 $\mu\text{g/L}$). (76) Moreover, a proportion of normalization of GH secretion in adulthood has been demonstrated as high as 87% for children with isolated IGHD of childhood onset. (75)

Additionally, early retesting of children diagnosed as idiopathic GHD with a normal MRI has turned out to normalize in up to 85% in 1-6 months after diagnosis (95), and has been recommended. (96)

The reasons for the high proportion of patients who are apparently increasing their GH secretion at retesting are however likely to be multifactorial. Long-term exposure to sex steroids may increase pituitary size and GH secretion, some children may have had a form of transient GHD and, eventually, some others may have been incorrectly diagnosed as GHD, given the problems associated with biochemical testing outlined previously.

3. Congenital Hypogonadotropic Hypogonadism

3.A DEFINITION

Congenital Hypogonadotropic Hypogonadism (cHH) is an organic and heterogeneous disease due to abnormal secretion or action of GnRH. It may be characteristically associated with an olfactory defect such as anosmia/hyposmia, the so called Kallmann syndrome (KS), or may occur in the context of normal olfaction, also known as normosmic HH (nHH). (2) The possible association with olfactory defects is related to the common embryonic origins of the GnHR-secreting and olfactory neurons, which both migrate from the olfactory placode to their final location in the brain through the cribriform plate of the ethmoid bone.

cHH is defined by the criteria of a prepubertal state at the age of >14 years and by a testicular volume not reaching a value of ≥ 5 mL during a 24-month follow-up despite a short trial with testosterone. A final differential diagnosis between CDGP is made if spontaneous puberty does not occur by the age of 18 years old. (44) However, the wait-and see approach is clearly not recommended because of the known psychosocial and medical issues associated with a pubertal delay already cited above.

3.B PREVALENCE

cHH is a rare disease with a frequency of 1:4000-6000 males with a 3-5:1 male:female sex ratio in tertiary centers. (97) The prevalence worldwide is difficult to determine, but it has been reported spanning from 1:10.000 to around 1: 86.0000, but when interpreting these findings methodological limitations of case ascertainment by medical record review should be kept in mind. (98) cHH may be familial or sporadic.

3.C CLINICAL PRESENTATION

A wide spectrum of phenotypes have been described.

- Complete cHH starting from foetal/neonatal life
- Complete cHH starting from the peripubertal period
- Partial cHH with an initial spontaneous drive of pubertal development but a subsequent sudden arrest (one third of the cases)
- Same as above with a reversal of GnRH secretion during adulthood (almost 10% of the cases)
- Adult onset cHH

The phenotype may vary accordingly. In boys the presence of microphallus, mild hypospadias and/or (bilateral) cryptorchidism may indicate a complete defect of foetal/neonatal origin, whereas the absence of these signs but the lack of puberty to start or to progress till Tanner 5 may indicate either a complete peripubertal form or a partial form of cHH, respectively, which may be difficult to differentiate from self-limiting constitutional delay. In females the scenario is even less well-defined, since the foetal/neonatal phenotype of cHH females is still unknown and possibly indistinguishable from other phenotypes. Clinical features of cHH of peripubertal onset or of partial forms in females are again the absence/ the incomplete self progressing puberty and primary amenorrhea, both overlapping with CDGP females, and the presence of eunuchoid proportions. (97) Interestingly, in both sexes, gonadarche alone is found to be absent, whereas adrenarche and pubarche may be present.

(25)

It is therefore clear that a complete physical examination considering limbs' asymmetry (ratio arm span/standing height > 1 , arm span > 6 cm of standing height and for eunuchoid proportions, sitting height/leg length ratio $< 0.8-1$) may orient clinicians towards cHH when disproportionateness is clinically detectable, taking available clinical chart for normal children as a reference. (99)

Short stature at pubertal age in cHH accounts for 40% of the cases, further rising complexity to the picture, however, an accurate analysis of the patient's growth chart may help in the diagnosis. In boys with cHH, in fact, height SDS does not differ to that of target potential till the first 5 years of life but a typical decrease at puberty occurs, with an inverse correlation with BMI, which is frequently found to be in the higher part of normal range. (30) Similar data on growth charts are however lacking in literature for cHH girls.

Apart from these specific endocrine phenotypes, other specific features, both neurological and extra-neurological, may be present. Associated neurological phenotypes, include a defective smell sensation, sensorineural hearing loss, color blindness (daltonism), nystagmus and bimanual synkinesis (mirror movements). Hypo-Anosmia, that can be found in 50% of the patients, is also a pathognomonic sign of Kallman syndrome and may help in the diagnostic process. (2) The olfactory competence can be quantitatively tested with specific tests e.g. the University of Pennsylvania Smell Identification test (UPSIT) or the Brief-Smell Identification test (B-SIT). This evaluation is critical since many patients with cHH and hypo-anosmia are not aware of their defective sense of smell.

Other non-neurological signs are also described, such as midline defects (cleft lip/palate; arched palate), dental abnormalities, renal agenesis/dysgenesis, syndactyly, polydactyly, camptodactyly, and metacarpal shortness. All of these features have to be carefully elicited during the physical examination of these patients and need to be considered all together because some of them can concomitate in GHD patients (**Figure 2**).

Moreover, a deep clinical examination must rule out syndromic conditions associated with hypogonadotropic hypogonadism. For example, the presence of morbid obesity and mental retardation should suggest Prader Willy Syndrome or Bardet-Biedl syndrome. The combination of coloboma, heart malformations, choanal atresia, growth retardation, genital anomalies should point out a diagnosis of CHARGE syndrome. The presence of 46 XY sex reversal and primary adrenal insufficiency should suggest adrenal hypoplasia congenita secondary to NR0B1 mutation. The presence of other pituitary deficiencies and midline defects should suggest Septo Optic Dysplasia, or the rare Hartsfield syndrome or Gordon Holmes syndrome. (19) (97) The presence of ulnar forearm abnormalities, absence/hypoplasia of mammary and apocrine glands eventually associated with midline defects (i.e. hypoplastic anterior pituitary) or renal malformations in the proband or in a family member may suggest UMS. (31) (**Figure 2**)

3.D ENDOCRINE WORKUP

Basal gonadotropins levels are in the majority of the cases not discriminative. For this reason, other basal endocrine markers had been investigated. Inhibin B, a protein derived from gonadal tissues through FSH stimulation (seminiferous tubules of the testis or cortical layer of the ovaries), are thought to be lower in cHH than in other conditions because of the absence of minipuberty in these patients able to trigger the differentiation either of Sertoli cells in males either of Granulosa cells in females. In males, a low Inhibin B level (< 35 pg/mL) (40), or the combination of low Inhibin B levels (10-49 pg/mL) together with low testis volume (< 1 mL) (22), seems to discriminate at best (100% sensitivity and specificity) cHH patients. Moreover, the combination of a low basal LH (< 0.3 IU/L) and a low basal Inhibin B (< 111 pg/ml) has been proven to provide 100% sensitivity and 98% specificity for the cHH diagnosis, giving a valid and reliable alternative to dynamic and more invasive stimulations. (100) In females with cHH, Inhibin B levels are however reported to be either low or normal compared to age-matched controls, therefore not discriminative. (42)

Stimulation test may also be helpful in the diagnostic process. GnRHa Triptorelin proved to have an excellent accuracy for the diagnosis of cHH if LH peaks prove to be lower than 5.3 U/L after 4 hours of pharmacological stimulation (100% sensibility and specificity). (100)

The hCG stimulation test has also been used for many years, primarily to assess the presence of functioning testicular tissue and to investigate defects of testosterone biosynthesis and action. It is based on the ability of hCG to increase androgen production in Leydig cells via stimulation of the LH receptor. Therefore, a blunted testosterone response to hCG stimulation test has been supposed as evidence towards a cHH diagnosis. However, many protocols have been used and large studies are lacking. Promising results have been assumed with longer protocols (19 days of stimulation proving to perform better than 3 days) (45) or higher doses (subsequent injections of 1500 UI of hCG in three consecutive days) (46). Combining the results of GnRH test stimulation and hCG test has also proved to differentiate cHH from CDGP with a sensibility and a specificity of 100% if LH peaks under LHRH test are < 2.8 U/L, testosterone levels after 3 day hCG test are < 3.6 nmol/L and testosterone levels after 19 days of hCG test are < 9.5 nmol/L. (45)

3.E GENETIC TESTING

The pathogenesis of cHH, with or without hypo/anosmia, is a wide area of current research in endocrinology. A genetic basis of cHH can be defined in almost 50% of the cases. (97) The genetic heterogeneity of cHH is due to the fact that all modes of inheritance can occur: X-linked, autosomal recessive and autosomal dominant. Rising complexity to the picture, the traditional Mendelian view of inheritance has been revised following identification of oligogenic forms of cHH in at least 20% of the cases. (2) Moreover, a specific genotype-phenotype correlation is frequently lacking, as the penetrance is incomplete and the expressivity extremely variable, again possibly due to the oligogenic fashion of inheritance, although an involvement of environmental factors cannot be excluded. Many

mutations in genes known to cause aberrations of the hypothalamic-pituitary-gonadal axis have been described. The mutations can be classified as either affecting GnRH action, GnRH secretion, GnRH neurons development and migration or GnRH neurons specification and/or activation, and many of them are shared with CDGP cohorts. Moreover, some mutation in genes responsible for hereditary forms of MPHD or syndromic conditions have also been associated with both cHH and GHD. (97) The genetic basis of cHH and shared causes with CDGP or GHD cohort are illustrated in **Figure 1**.

3.F FINAL OUTCOMES

For males, treatment is based on the use of testosterone esters given either by intramuscular or transdermal routes with subsequent gradual dose increase every 6 months until reaching, in around 36 months, the adult scheme. The use of testosterone, either injectable or transdermal, leads to good virilization but has little-no effect on testicular growth. Treatment for females with hypogonadism is based on the initial use of estrogens in the form of 17 β -estradiol (preferably), either trans dermally (fancied) or orally, or in the form of oral EE. Estrogen therapy must also be integrated with progestin therapy after at least 2 years (or earlier in the case of more than one significant bleeding episode) in order to avoid endometrial hypertrophy. The natural (i.e. micronized) over the synthetic forms of progestins are preferred. A complete explanation of therapeutic protocols and doses is beyond the scope of this review but can be found in the recent review published by Raivio T. et al. (3) While testosterone replacement only causes virilization, treatment with recombinant FSH associated with hCG has been shown to induce testes growth, increase Inhibin B levels and stimulate spermatogenesis in adolescent males with cHH. Moreover, an early treatment with gonadotropins has been suggested to have a positive effect on future fertility. (44) Also, as already mentioned, the observation of a self limiting pubertal progression at discontinuation of sex steroids therapy may point out a cHH rather than CDGP diagnosis. (3)

EVIDENCE SYNTHESIS

In every child referred for a suspected growth disorder, anthropometric measurements, medical history (with special attention to the so called “red flag”, see **Figure 2**), full physical examination with special attention to dysmorphisms and disproportions, pubertal development and bone age assessment must be evaluated by the pediatric endocrinologist.

Regarding auxological measurements standing height, arm span, sitting height and leg length must be measured at every examination, with the use of a proper stadiometer and a tape-measure. At the same time pubertal stage must be evaluated by the use of Tanner charts, with the use of a proper orchidometer for boys. At this regard it is worth remembering that, even though in both sexes hair growth in the pubic area depends on adrenal secretion, clinically, a delayed gonadarche together with a delayed adrenarche and pubarche orients towards a CDGP diagnosis, whereas delayed gonadal development alone is a more peculiar presentation of cHH patients. (25)

Bone age is another important diagnostic tool. To this extent it is worth remembering that in every child, TW3 method and G&P method may overestimate and underestimate bone age, respectively. (28) Moreover, because G&P standards and TW standards were derived from white children living in the United States and predominantly of North European ancestry, the use of new automated methods is recommended for the assessment of children of other ethnicity groups. (27) Bone age is usually found to be delayed in relation to chronological age in all three CDGP, GHD and cHH patients. Even if many endocrine (hypothyroidism, rickets, Cushing disease, panhypopituitarism) and non-endocrine conditions (malnutrition, prematurity, cardiac, liver or kidney diseases, systemic inflammations or infections) may present with a delayed bone age, this assessment is considered a non-invasive and cost-effective method able to select those patients in which further testing are recommended. In fact, a great proportion of patients with non-endocrine short stature (familial short stature, skeletal dysplasia, idiopathic short stature) may be excluded from further testing because

1 displaying a bone age equal or sometimes advanced compared to chronological age, or because
2
3 manifesting straightforward signs of skeletal dysplasia on X-rays. Moreover, usually, a failure of
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5 spontaneous pubertal development at the bone age maturation of 12 and 13 years, in females and
6
7 males respectively, may be indicative of an organic cause rather than CDGP. (28)
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10 Bone age is also a useful starting point to predict FH outcomes. (**Table II**) PAH may be helpful for
11
12 the differential diagnosis, as it will end up laying in the lower end of target height for CDGP patients,
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14 be far way lower than TH in GHD children, hence higher than TH in cHH patients. (**Table I**).
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18 Taken as a whole, self-limited delayed puberty is the most common cause of short stature and delayed
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20 puberty in both sexes in clinical practice, and the higher number of males that present to medical
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22 attention is thought to be a consequence of referral bias. According to the literature, we can expect to
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24 find an underlying secondary medical cause of poor growth/delayed puberty in up to 27% of boys
25
26 and 57% of girls presenting to medical attention. (3) Chronic systemic illnesses and malnutrition (the
27
28 so called “functional causes” of delayed puberty) and other causes of poor growth must be ruled out
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30 by baseline blood exams including full blood count, liver function test, creatinine, celiac screen,
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32 thyroid function tests, IGF-1, PRL, LH/FSH, testosterone/estradiol levels. (25) At the same time, the
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34 evidence of syndromic stigmata may evoke a specific syndromic phenotype or a contiguous gene
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36 syndrome. The targeted genome analysis and/or a standard karyotype together with an array-CGH
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38 may be useful investigations in these situations. When all these confounding phenotypes are ruled
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40 out, the diagnosis must be carried out through a step-by-step approach (**Figure 3**).
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45 Useful information can firstly be taken from growth charts from infancy, at least in boys. CDGP boys
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47 soon after birth are known to display poor growth until 2 years old, then tend to level till 5 years old,
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49 starting from when a height SDS loss starts to be detectable reaching their nadir at puberty (26) and
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51 BMI is found to be concordantly low. (30) By contrast, in boys with GHD a continuous decrease in
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53 height SDS without any levelling is found from birth on and a severe short stature is frequently
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detected (68), together with an increase in fat mass (in patients left untreated). (30) In boys with cHH, instead, height SDS does not differ to that of target potential in the first 5 years of life but a typical decreases at puberty occurs, with an inverse correlation with BMI. (30) Special considerations must then be taken for girls, in which GV does not discriminate at best the different etiologies of delayed puberty. (22)

If cHH presents with pathognomonic “red flags” (e.g. anosmia, eunuchoid habitus, pituitary lesions, ulnar-mammary malformations) this should help in the diagnostic process, however many features seem to overlap with GHD cohorts, further mistaking the diagnosis (**Figure 2**).

Anyway, not all peripubertal children with pathological causes of growth failure present to medical attention with short stature. If insufficient clues for a disturbed growth are found at the first screening, the patient can be put on watchful waiting. If clues for disturbed growth are highlighted, additional further investigations must be performed. A combination of short stature ($\text{SDS H} < -2.5$), severe distance to target height ($\Delta \text{SDS H} - \text{SDSTH} > -1.6$) and growth deflection ($>0.5 \text{ SDS/year}$ or $> 0.7 \text{ SDS/2 years}$ or $> 1 \text{ SDS/undefined time}$) should be used as practical guideline to decide which child should be screened with a GHST (17)

An important consideration must be taken for those children in which puberty may be anticipated: in this scenario growth velocity may be falsely reassuring because driven by pubertal hormones, but then a severe deviation from target height becomes evident after puberty completion if growth hormone deficiency concomits. For this reason patients at risk of having GHD (i.e. Cancer Survivors treated with surgery and/or total body radiation and/or chemotherapy) must be investigated with a growth hormone stimulation test regardless of height SDS and GV SDS. (33)

In terms of GHST, hypothalamic stimulation (hypoglycemia tolerance test, clonidine test, arginine test, glucagon test) are preferred rather than combined stimuli (i.e. GHRH+ arginine) in order to rule out possible hypothalamic causes of growth hormone deficiency. Moreover, priming with sex steroids

before GHST is useful in order to increase diagnostic accuracy of the stimulation and reduce the false positive diagnosis of presumed peripubertal idiopathic GHD, at least for boys > 11 years old and for girls > 10 years old. (32) To this extent many protocols exist within different centers (39): the use of 100 mg of testosterone enanthate intramuscularly 1 week before the test for boys, and 1-2 mg (< 20 kg or > 20 kg respectively) of valerate estradiol orally for girls for the three nights before the test may be considered a reasonable scheme, even though oral estradiol preparations can also be used for boys if a less invasive route of administration is preferred. If GH peak under GHST is less than 7-8 $\mu\text{g/L}$ (with immunometric assays), especially under 2 different stimulations, the child is considered as having GHD and a pituitary MRI is warranted. If no tumoral lesions in the brain are found, rhGH therapy can be started at substitutive doses (25-35 $\mu\text{g/kg/die}$) and the child must be followed up periodically in order to verify appropriate catch-up growth after rhGH is commenced. According to the severity of GHD and to the underlying cause, early retesting of GH secretion may be considered. (95) (96)

It is also worth mentioning that in terms of final height outcomes a lot of different results are described in literature for treated GHD patients, showing a FH SDS spanning between -1.1 to -0.5 and ΔSDS FH -TH concordantly ranging between -0.6 to +1.08. For this reason, it is also very difficult to confirm the diagnosis backwards, as these results overlap mostly with those described in some CDGP cohorts (**Tables III and IV**). This is possibly due to the low specificity of GHST per se together with the fact that most of these studies were performed without any priming preparation before stimulation, and as a result they might include also some false positive patients that could have possibly displayed normal GH peaks if sex steroids priming would have been performed.

If GH peaks prove instead to be higher than 7-8 $\mu\text{g/L}$ in at least one GHST, a diagnosis of CDGP or cHH may be expected. The differential diagnosis between these two conditions is however far from being straightforward, especially in the peripubertal age given the heterogeneity of these diseases and the tendency to overlap of one into another clinically, hormonally and genetically. (**Figures 1 and 2**).

Basal gonadotropin levels have limited diagnostic specificity as they tend to be low both in CDGP and cHH cohorts. Even if in some CDGP patients at the very beginning of puberty gonadotropins may be detectable ($LH > 0.2-0.3$ U/L), this picture again tends to overlap at least with partial forms of cHH. (43)

For this reason, dynamic stimulation of HPG axis has been proposed. A combination of LHRH test and hCG test (3days & 19 days stimulation) has been proposed in order to increase sensitivity and specificity of cHH diagnosis by 100%. (45)

At least in boys, Sertoli cell markers but not Leydig cells, growth or adrenal markers are considered helpful in establishing whether a boy with delayed puberty will progress spontaneously. (41) Inhibin B may provide a simple first-line test capable of identifying a subset of patients with delayed puberty who are highly likely to have cHH. These test may be performed before a short period of treatment with LDSS, which is considered more indicative and helpful. In fact, an increase in growth velocity and in gonadal and breast development after a short course of 3-6 months of LDSS can be taken as evidence against the presence of GHD or cHH. (3) (64) This treatment may at the same time be helpful for CDGP children in sensibilizing their anterior pituitary glands to sex steroids in order to allow puberty to trigger spontaneously, minimizing psychosocial distress of these patients compared to peers and avoiding the known long term metabolic and bone sequelae associated with delayed puberty. (2)

Finally, it is worth mentioning that as these conditions may overlap mostly in terms of genetic causes, this is not yet a field able to differentiate these conditions, even though some promising studies on specific genes selectively discovered in CDGP families have been recently published. (19)

CONCLUSIONS

After systemic chronic illnesses or other causes of “functional” delayed growth have been ruled out, CDGP may be the most probable cause of poor growth in a child presenting with short stature and delayed puberty, especially for boys. However the differential diagnosis between CDGP, GHD and cHH is a challenging one for the clinician at the beginning of clinical examination, for many reasons. First, as already mentioned, children may present with the same auxological features (short stature, low growth velocity, delayed puberty, delayed bone age). Secondary, even if PAH may help, it is difficult to reliably trust the predicted adult height given the low accuracy of different methods, especially if bone age is extremely delayed. Third, although the presence of a positive familial history of delayed puberty may give a cue towards a presumptive CDGP diagnosis, cHH cannot be excluded giving the high heterogeneity of cHH presentation within pedigrees. Fourth, because of the lack of sensibilization of the pituitary gland of these children to detectable levels of sex steroids, peripubertal CDGP can display decreased GH stimulation peaks under GHST and lower IGF-1 levels compared with their pubertal, age-matched peers, pointing out the need of further investigations in order to rule out a low pituitary growth hormone reserve. For this reason we recommend the use of priming before GHST in every patient tested for GHD at least from the age of 10 years old (girls) or 11 years old (boys). A treatment hormonal approach is also recommended afterwards for those patients with normal GH peaks under GHST in order to allow a correct differential diagnosis between CDGP and cHH, being beneficial at the same time for psychosocial wellness, final height outcomes, sexual, bone and metabolic health.

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50
51
52
53
54
55

REFERENCES

1. Barinaga M, Yamonoto G, Rivier C, Vale W, Evans R, Rosenfeld MG. Transcriptional regulation of growth hormone gene expression by growth hormone-releasing factor. *Nature* 1983; 306(5938): 84–5.
2. Boehm U, Bouloux P-M, Dattani MT, de Roux N, Dodé C, Dunkel L, et al. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism--pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* 2015;11(9):547–64.
3. Raivio T, Miettinen PJ. Constitutional delay of puberty versus congenital hypogonadotropic hypogonadism: Genetics, management and updates. *Best Pract Res Clin Endocrinol Metab* 2019;33(3):1013–16.
4. Weissberger AJ, Ho KK. Activation of the somatotrophic axis by testosterone in adult males: evidence for the role of aromatization. *J Clin Endocrinol Metab* 1993;76(6):1407–12.
5. Veldhuis JD, Metzger DL, Martha PM, Murras N, Kerrigan JR, Keenan B, et al. Estrogen and testosterone, but not a nonaromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. *J Clin Endocrinol Metab* 1997;3414–20.
6. Meinhardt UJ, Ho KKY. Modulation of growth hormone action by sex steroids. *Clin Endocrinol (Oxf)* 2006; 65(4):413–22.

- 1 7. Hartmann BW, Huber JC, Kirchengast S, Söregi, M.D. G, Albrecht AE. Effect of
2 hormone replacement therapy on growth hormone stimulation in women with
3 premature ovarian failure. *Fertil Steril* 1997; 68(1):103–7.
- 4
5
6
7
8 8. González-Parra S, Argente J, García-Segura LM, Chowen JA. Cellular Composition
9 of the Adult Rat Anterior Pituitary Is Influenced by the Neonatal Sex Steroid
10 Environment. *Neuroendocrinology* 1998;68(3):152–62.
- 11
12
13
14
15
16 9. Yan M, Jones MEE, Hernandez M, Liu D, Simpson ER, Chen C. Oestrogen
17 Replacement in vivo Rescues the Dysfunction of Pituitary Somatotropes in
18 Ovariectomised Aromatase Knockout Mice. *Neuroendocrinology* 2005;81(3):158–66.
- 19
20
21
22
23
24 10. Murras N, Rogol AD, Haymond MW, Veldhuis JD. Sex steroids, growth hormone,
25 insulin-like growth factor-1: neuroendocrine and metabolic regulation in puberty.
26 *Horm Res* 1996;45(1–2):74–80.
- 27
28
29
30
31 11. Albertsson-Wikland K, Rosberg S, Libre E, Lundberg LO, Groth T. Growth hormone
32 secretory rates in children as estimated by deconvolution analysis of 24-h plasma
33 concentration profiles. *Am J Physiol Metab.* 1989 Dec;257(6):E809–14.
- 34
35
36
37
38 12. Giustina A, Scalvini T, Tassi C, Desenzani P, Poiesi C, Wehrenberg WB, et al.
39 Maturation of the regulation of growth hormone secretion in young males with
40 hypogonadotropic hypogonadism pharmacologically exposed to progressive
41 increments in serum testosterone. *J Clin Endocrinol Metab.* 1997;82(4):1210–9.
- 42
43
44
45
46
47 13. Mansfield MJ, Rudlin cCR, Crigler JF, Karol KA, Crawford JD, Boepple PA, et al.
48 Changes in Growth and Serum Growth Hormone and Plasma Soipatomedin-C
49 Levels During Suppression of Gonadal Sex Steroid Secretion in Girls with Central
50 Precocious Puberty. *J Clin Endocrinol Metab* 1988 Jan;66(1):3–9.
- 51
52
53
54
55

- 1 14. Marin G, Domené HM, Barnes KM, Blackwell BJ, Cassorla FG, Cutler GB. The
2 effects of estrogen priming and puberty on the growth hormone response to
3 standardized treadmill exercise and arginine-insulin in normal girls and boys. *J Clin*
4 *Endocrinol Metab* 1994;79(2):537–41.
5
6
7
8
9
- 10 15. Wit JM, Ranke MB KC. International Classification of Paediatric Endocrine
11 Diagnoses. *Hormon Res. Pediatr.* 2016; 86(3): 212-214.
12
13
14
15
- 16 16. Lawaetz JG, Hagen CP, Mieritz MG, Jensen MB, Petersen JH, Juul A. Evaluation of
17 451 Danish boys with delayed puberty: Diagnostic use of a new puberty nomogram
18 and effects of oral testosterone therapy. *J Clin Endocrinol Metab* 2015;100(4):1376–
19 85.
20
21
22
23
24
- 25 17. Stalman SE, Hellinga I, Wit JM, Hennekam RCM, Kamp GA, Plötz FB. Growth
26 failure in adolescents: etiology, the role of pubertal timing and most useful criteria for
27 diagnostic workup. *J Pediatr Endocrinol Metab* 2016;29(4):465–73.
28
29
30
31
32
- 33 18. Cassatella D, Howard SR, Acierno JS, Xu C, Papadakis GE, Santoni FA, et al.
34 Congenital hypogonadotropic hypogonadism and constitutional delay of growth and
35 puberty have distinct genetic architectures. *Eur J Endocrinol* 2018;178(4):377–88.
36
37
38
39
- 40 19. Howard SR. The genetic basis of delayed puberty, *Frontiers in Endocrinology*.
41 *Frontiers Media S.A.* 2019; (10): 423.
42
43
44
45
- 46 20. Reindollar RH, Rogers Byrd J, McDonough PG. Delayed sexual development: A
47 study of 252 patients. *Am J Obstet Gynecol* 1981;140(4):371–80.
48
49
50
51
- 52 21. Grimberg A, Feemster KA, Pati S, Ramos M, Grundmeier R, Cucchiara AJ, et al.
53 Medically underserved girls receive less evaluation for short stature. *Pediatrics*.
54 2011;127(4):696–702.
55

22. Varimo T, Miettinen PJ, Käsäkoski J, Raivio T HM. Congenital hypogonadotropic hypogonadism, functional hypogonadotropism or constitutional delay of growth and puberty? An analysis of a large patient series from a single tertiary center. *Hum Reprod* 2017;32(1):147–53.
23. Wudy SA, Hagemann S, Dempfle A, Ringler G, Blum WF, Berthold LD, et al. Children with idiopathic short stature are poor eaters and have decreased body mass index. *Pediatrics* 2005; 116(1):52-7.
24. Han JC, Balagopal P, Sweeten S, Darmaun D, Mauras N. Evidence for hypermetabolism in boys with constitutional delay of growth and maturation. *J Clin Endocrinol Metab*. 2006;91(6):2081–6.
25. Howard SR, Dunkel L. Delayed Puberty—Phenotypic Diversity, Molecular Genetic Mechanisms, and Recent Discoveries. *Endocr Rev*. 2019;40(5):1285–317.
26. Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. *J Clin Endocrinol Metab* 2002;87(4):1613–20.
27. Ren X, Li T, Yang X, Wang S, Ahmad S, Xiang L, et al. Regression Convolutional Neural Network for Automated Pediatric Bone Age Assessment from Hand Radiograph. *IEEE J Biomed Heal Informatics* 2019;23(5):2030–8.
28. Creo AL, Schwenk WF. Bone age: A handy tool for pediatric providers *Pediatrics*. American Academy of Pediatrics 2017; Vol. 140
29. Brämswig JH, Fasse M, Holthoff M-L, von Lengerke HJ, von Petrykowski W, Schellong G. Adult height in boys and girls with untreated short stature and constitutional delay of growth and puberty: Accuracy of five different methods of height prediction. *J Pediatr* 1990;117(6):886–91.

- 1 30. Reinehr T, Hoffmann E, Rothermel J, Lehrian TJ, Brämwig J, Binder G. A New
2 Model of Adult Height Prediction Validated in Boys with Constitutional Delay of
3 Growth and Puberty. *Horm Res Paediatr* 2019;91(3):186–94.
4
5
6
7
8
9 31. Galazzi E, Duminuco P, Moro M, Guizzardi F, Marazzi N, Sartorio A, et al.
10 Hypogonadotropic hypogonadism and pituitary hypoplasia as recurrent features in
11 ulnar-mammary syndrome. *Endocr Connect*. 2018;7(12) 1432-1441.
12
13
14
15
16 32. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, et al.
17 Guidelines for Growth Hormone and Insulin-Like Growth Factor-I Treatment in
18 Children and Adolescents: Growth Hormone Deficiency, Idiopathic Short Stature,
19 and Primary Insulin-Like Growth Factor-I Deficiency. *Horm Res Paediatr*. 2017;86(6)
20 361–97.
21
22
23
24
25
26
27 33. Collett-Solberg PF, Ambler G, Backeljauw PF, Bidlingmaier M, Biller BMK,
28 Boguszewski MCS, et al. Diagnosis, Genetics, and Therapy of Short Stature in
29 Children: A Growth Hormone Research Society International Perspective. *Horm Res*
30 *Paediatr*. 2019;92(1):1–14.
31
32
33
34
35
36 34. Martínez AS, Domené HM, Ropelato MG, Jasper HG, Pennisi PA, Escobar ME, et
37 al. Estrogen Priming Effect on Growth Hormone (GH) Provocative Test: A Useful
38 Tool for the Diagnosis of GH Deficiency. *J Clin Endocrinol Metab*.
39 2000;85(11):4168–72.
40
41
42
43
44
45 35. Molina S, Paoli M, Camacho N, Arata-Bellabarba G, Lanes R. Is Testosterone and
46 Estrogen Priming Prior to Clonidine Useful in the Evaluation of the Growth Hormone
47 Status of Short Peripubertal Children? *J Pediatr Endocrinol Metab*. 2008;21(3) 257-
48 66.
49
50
51
52
53
54 36. Müller G, Keller A, Reich A, Hoepffner W, Kratzsch J, Buckler JM, et al. Priming with
55

Testosterone Enhances Stimulated Growth Hormone Secretion in Boys with Delayed Puberty. *J Pediatr Endocrinol Metab* 2004;17(1) 77-83.

37. Soliman A, Adel A, Sabt A, Elbukhari E, Ahmed H, Sanctis V. Does priming with sex steroids improve the diagnosis of normal growth hormone secretion in short children? *Indian J Endocrinol Metab* 2014;18(7):80-3.
38. Gonc EN, Kandemir N, Ozon A, Alikasifoglu A. Final heights of boys with normal growth hormone responses to provocative tests following priming. *J Pediatr Endocrinol Metab* 2008;21(10):963–71.
39. Murray PG, Dattani MT, Clayton PE. Controversies in the diagnosis and management of growth hormone deficiency in childhood and adolescence *Archives of Disease in Childhood*. 2016;101,96–100.
40. Coutant R, Biette-Demeneix E, Bouvattier C, Bouhours-Nouet N, Gatelais F, Dufresne S, et al. Baseline inhibin B and anti-Mullerian hormone measurements for diagnosis of hypogonadotropic hypogonadism (HH) in boys with delayed puberty. *J Clin Endocrinol Metab* 2010;95(12):5225–32.
41. Rohayem J, Nieschlag E, Kliesch S, Zitzmann M. Inhibin B, AMH, but not INSL3, IGF1 or DHEAS support differentiation between constitutional delay of growth and puberty and hypogonadotropic hypogonadism. *Andrology* 2015;3(5):882–7.
42. Li HWR, Anderson RA, Yeung WSB, Ho PC, Ng EHY. Evaluation of serum antimullerian hormone and inhibin B concentrations in the differential diagnosis of secondary oligoamenorrhea. *Fertil Steril* 2011;96(3):774–9.
43. Resende EAMR, Lara BHJ, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of Basal and Gonadotropin-Releasing Hormone-Stimulated

Gonadotropins by Immunochemiluminometric and Immunofluorometric Assays in Normal Children. *J Clin Endocrinol Metab* 2007;92(4):1424–9.

44. Harrington J, Palmert MR. Clinical review: Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. *J Clin Endocrinol Metab* 2012. 97 (9) 3056–67.
45. Segal TY, Mehta A, Anazodo A, Hindmarsh PC, Dattani MT. Role of Gonadotropin-Releasing hormone and human chorionic gonadotropin stimulation tests in differentiating patients with hypogonadotropic hypogonadism from those with constitutional delay of growth and puberty. *J Clin Endocrinol Metab* 2009;94(3):780–5.
46. Kauschansky A, Dickerman Z, Phillip M, Weintrob N, Strich D. Use of GnRH agonist and human chorionic gonadotrophin tests for differentiating constitutional delayed puberty from gonadotrophin deficiency in boys. *Clin Endocrinol (Oxf)* 2002. 56(5) 603–7.
47. Chan YM, Lippincott MF, Kusa TO, Seminara SB. Divergent responses to kisspeptin in children with delayed puberty. *JCI insight*. 2018;3(8) e99109.
48. Dunkel L, Quinton R. Transition in endocrinology: Induction of puberty. Vol. 170, *European Journal of Endocrinology*. BioScientifica Ltd.; 2014 R229-39.
49. Sukumar SP, Bhansali A, Sachdeva N, Ahuja CK, Gorski U, Jarial KDS, et al. Diagnostic utility of testosterone priming prior to dynamic tests to differentiate constitutional delay in puberty from isolated hypogonadotropic hypogonadism. *Clin Endocrinol (Oxf)* 2017;86(5):717–24.
50. Taha D, Mullis PE, Ibáñez L, De Zegher F. Absent or delayed adrenarche in Pit1/

POU1F1 deficiency. *Horm Res* 2005; 64(4):175–9.

51. Pugliese-Pires PN, Fortin JP, Arthur T, Latronico AC, Mendonca BB, Villares SMF, et al. Novel inactivating mutations in the GH secretagogue receptor gene in patients with constitutional delay of growth and puberty. *Eur J Endocrinol* 2011;165(2):233–41.
52. Day FR, Elks CE, Murray A, Ong KK, Perry JRB. Puberty timing associated with diabetes, cardiovascular disease and also diverse health outcomes in men and women: The UK Biobank study. *Sci Rep.* 2015;(5):11208.
53. Bourguignon JP. Variations in duration of pubertal growth: a mechanism compensating for differences in timing of puberty and minimizing their effects on final height. Belgian Study Group for Paediatric Endocrinology. *Acta Paediatr Scand Suppl* 1988;347:16–24.
54. Crowne EC, Shalet SM, Wallace WH, Eminson DM, Price DA. Final height in boys with untreated constitutional delay in growth and puberty. *Arch Dis Child* [Internet]. 1990;65(10):1109–12.
55. Poyrazoğlu Ş, Günöz H, Darendeliler F, Saka N, Bundak R, Baş F. Constitutional delay of growth and puberty: From presentation to final height. *J Pediatr Endocrinol Metab.* 2005;18(2):171–9.
56. Couto-Silva AC, Trivin C, Adan L, Lawson-Body E, Souberbielle JC, Brauner R. Management of boys with short stature and delayed puberty. *J Pediatr Endocrinol Metab* 2005;18(8):807–13.
57. Zucchini S, Wasniewska M, Cisternino M, Salerno M, Iughetti L, Maghnie M, et al. Adult height in children with short stature and idiopathic delayed puberty after

different management. Eur J Pediatr 2008 Jun 24;167(6):677–81.

58. Martin MM, Martin ALA, Mossman KL. Testosterone treatment of constitutional delay in growth and development: effect of dose on predicted versus definitive height. Acta Endocrinol (Copenh) 1986;113(4_Suppl):S147–52.
59. Uruena M, Pantiotou S, Preece MA, Stanhope R. Is testosterone therapy for boys with constitutional delay of growth and puberty associated with impaired final height and suppression of the hypothalamo-pituitary-gonadal axis? Eur J Pediatr 1992 J;151(1):15–8.
60. Kohva E, Varimo T, Huopio H, Tenhola S, Voutilainen R, Toppari J, et al. Anti-Müllerian hormone and letrozole levels in boys with constitutional delay of growth and puberty treated with letrozole or testosterone. Hum Reprod 2020;35(2):257–64.
61. Stanhope R, Buchanan CR, Fenn GC, Preece MA. Double blind placebo controlled trial of low dose oxandrolone in the treatment of boys with constitutional delay of growth and puberty. Arch Dis Child 1988;63(5):501–5.
62. De Luca F, Argente J, Cavallo L, Crowne E, Delemarre-Van de Waal HA, De Sanctis C, et al. Management of Puberty in Constitutional Delay of Growth and Puberty. J Pediatr Endocrinol Metab 2001;14(s2):953–7.
63. Crowne EC, Shalet SM, Wallace WH, Eminson DM, Price DA. Final height in girls with untreated constitutional delay in growth and puberty. Eur J Pediatr 1991;150(10):708–12.
64. Kaplowitz PB. Diagnostic value of testosterone therapy in boys with delayed puberty. Am J Dis Child 1989;143(1):116–20.

- 1 65. Chioma L, Papucci G, Fintini D, Cappa M. Use of testosterone gel compared to
2 intramuscular formulation for puberty induction in males with constitutional delay of
3 growth and puberty: a preliminary study. *J Endocrinol Invest*. 2018 Feb 1;41(2):259–
4 63.
5
6
7
8
9
10 66. Overview | Human growth hormone (somatropin) for the treatment of growth failure
11 in children | Guidance | NICE. [Internet]. Available from
12 <https://www.nice.org.uk/guidance/TA188> [cited 2020 January 29]
13
14
15
16
17 67. Murray PG, Clayton PE. Disorders of Growth Hormone in Childhood. In: Feingold
18 KR, Anawalt B, Boyce A, et al., (Editors) *Endotext*. South Dartmouth (MA):
19 MDTText.com, Inc.; 2000. Available from:
20 <https://www.ncbi.nlm.nih.gov/books/NBK278971>
21
22
23
24
25
26 68. Rothermel J, Lass N, Toschke C, Reinehr T. Progressive decline in height standard
27 deviation scores in the first 5 years of life distinguished idiopathic growth hormone
28 deficiency from familial short stature and constitutional delay of growth. *Horm Res*
29 *Paediatr*. 2016;86(2):117–25.
30
31
32
33
34
35 69. Biczysko-Mokosa A, Petriczko E, Horodnicka-Józwa A, Dawid G, Kedzia A, Lewiński
36 A, et al. Evaluation of final height prediction and selected parameters in Polish
37 patients with severe and partial growth hormone deficiency. *Neuro Endocrinol Lett*.
38 2014;35(3):242–7.
39
40
41
42
43
44 70. Inoue-Lima TH, Vasques GA, Scalco RC, Nakaguma M, Mendonca BB, Arnhold IJP,
45 et al. IGF-1 assessed by pubertal status has the best positive predictive power for
46 GH deficiency diagnosis in peripubertal children. *J Pediatr Endocrinol Metab*. 2019
47 Feb 1;32(2):173–9.
48
49
50
51
52
53 71. Blum WF, Alherbish A, Alsagheir A, El Awwa A, Kaplan W, Koledova E, et al. The
54
55

growth hormone-insulin-like growth factor-I axis in the diagnosis and treatment of growth disorders. *Endocrine Connections*. BioScientifica Ltd. 2018;(7):R212–22.

72. Ghigo E, Bellone J, Aimaretti G, Bellone S, Loche S, Cappa M, et al. Reliability of provocative tests to assess growth hormone secretory status. Study in 472 normally growing children. *J Clin Endocrinol Metab* 1996;81(9):3323–7.
73. Giordano M. Genetic causes of isolated and combined pituitary hormone deficiency. *Best Pract Res Clin Endocrinol Metab* 2016;30(6):679–91.
74. Léger J, Danner S, Simon D, Garel C, Czernichow P. Do all patients with childhood-onset growth hormone deficiency (GHD) and ectopic neurohypophysis have persistent GHD in adulthood? *J Clin Endocrinol Metab* 2005;90(2):650–6.
75. Quigley CA, Zagar AJ, Liu CC, Brown DM, Huseman C, Levitsky L, et al. United States multicenter study of factors predicting the persistence of GH deficiency during the transition period between childhood and adulthood. *Int J Pediatr Endocrinol* 2013;(1):6.
76. Tauber M, Moulin P, Pienkowski C, Jouret B, Rochiccioli P. Growth hormone (GH) retesting and auxological data in 131 GH-deficient patients after completion of treatment. *J Clin Endocrinol Metab* 1997;82(2):352–6.
77. Toogood A, Shalet S. Diagnosis of severe growth hormone (GH) deficiency in young adults who received GH replacement therapy during childhood. *Acta Paediatr* 1997;86(S423):117–20.
78. Donaubauer J, Kiess W, Kratzsch J, Nowak T, Steinkamp H, Willgerodt H, et al. Re-assessment of growth hormone secretion in young adult patients with childhood-onset growth hormone deficiency. *Clin Endocrinol (Oxf)* 2003;58(4):456–63.

- 1 79. Murray PG, Hague C, Fafoula O, Gleeson H, Patel L, Banerjee I, et al. Likelihood of
2 persistent GH deficiency into late adolescence: relationship to the presence of an
3 ectopic or normally sited posterior pituitary gland. Clin Endocrinol (Oxf)
4 2009;71(2):215–9.
5
6
7
8
9
10 80. Yadav P, Singhal S, Chauhan S, Harit S. MRI evaluation of size and shape of
11 normal pituitary gland: age and sex related changes." J Clin Diagn Res 2017 TC01-
12 TC04.
13
14
15
16
17 81. Argyropoulou M, Perignon F, Brunelle F, Brauner R, Rappaport R. Height of normal
18 pituitary gland as a function of age evaluated by magnetic resonance imaging in
19 children. Pediatr Radiol 1991;21(4):247–9.
20
21
22
23
24 82. Han X, Xiu J, Huang Z, Zhang J, Zhang Z, Dong Y, et al. Three-dimensional
25 magnetic resonance volumetry of the pituitary gland is effective in detecting short
26 stature in children. Exp Ther Med 2014;8(2):551–6.
27
28
29
30
31
32 83. Doraiswamy PM, Potts JM, Axelson DA, Husain MM, Lurie SN, Na C, et al. MR
33 assessment of pituitary gland morphology in healthy volunteers: age- and gender-
34 related differences. AJNR Am J Neuroradiol;13(5):1295–9.
35
36
37
38
39 84. Kessler M, Tenner M, Frey M, Noto R. Pituitary volume in children with growth
40 hormone deficiency, idiopathic short stature and controls. J Pediatr Endocrinol
41 Metab 2016;29(10):1195–200.
42
43
44
45
46
47 85. Maghnie M, Lindberg A, Koltowska-Häggström M, Ranke MB. Magnetic resonance
48 imaging of CNS in 15043 children with GH deficiency in KIGS (Pfizer International
49 Growth Database). Eur J Endocrinol 2013;168(2):211–7.
50
51
52
53
54 86. Krström B, Aronson AS, Dahlgren J, Gustafsson J, Halldin M, Ivarsson SA, et al.
55

Growth Hormone (GH) Dosing during Catch-Up Growth Guided by Individual Responsiveness Decreases Growth Response Variability in Prepubertal Children with GH Deficiency or Idiopathic Short Stature. *J Clin Endocrinol Metab* 2009;94(2):483–90.

87. Reiter EO, Price DA, Wilton P, Albertsson-Wikland K, Ranke MB. Effect of growth hormone (GH) treatment on the near-final height of 1258 patients with idiopathic GH deficiency: analysis of a large international database. *J Clin Endocrinol Metab* 2006;91(6):2047–54.
88. Cutfield W, Lindberg A, Wikland KA, Chatelain P, Ranke M, Wilton P. Final height in idiopathic growth hormone deficiency: the KIGS experience. *Acta Paediatr* 1999;88(s428):72–5.
89. Blethen SL, Baptista J, Kuntze J, Foley T, LaFranchi S, Johanson A. Adult Height in Growth Hormone (GH)-Deficient Children Treated with Biosynthetic GH. *J Clin Endocrinol Metab* 1997;82(2):418–20.
90. Pozzobon G, Partenope C, Mora S, Garbetta G, Weber G, Barera G. Growth hormone therapy in children: predictive factors and short-term and long-term response criteria. *Endocrine* 2019; 66(3):614–621
91. Deodati A, Cianfarani S. Impact of growth hormone therapy on adult height of children with idiopathic short stature: systematic review. *BMJ* 2011;(342):c7157.
92. Wit JM, Ranke MB, Albertsson-Wikland K, Carrascosa A, Rosenfeld RG, Van Buuren S, et al. Personalized approach to growth hormone treatment: Clinical use of growth prediction models. Vol. 79, *Hormone Research in Paediatrics. Horm Res Paediatr*; 2013; 257–70.

93. Yuen KCJ, Biller BMK, Radovick S, Carmichael JD, Jasim S, Pantalone KM, et al. American association of clinical endocrinologists and american college of endocrinology guidelines for management of growth hormone deficiency in adults and patients transitioning from pediatric to adult care. *Endocr Pract* 2019;25(11):1191–232.
94. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, et al. Guidelines for Growth Hormone and Insulin-Like Growth Factor I Treatment in Children and Adolescents: Growth Hormone Deficiency, Idiopathic Short Stature, and Primary Insulin-Like Growth Factor-I Deficiency. *Horm Res Paediatr* 2016;86(6):361–97.
95. Loche S, Bizzarri C, Maghnie M, Faedda A, Tziella C, Autelli M, et al. Results of early reevaluation of growth hormone secretion in short children with apparent growth hormone deficiency. *J Pediatr* 2002;140(4):445–9.
96. Vuralli D, Gonc EN, Ozon ZA, Alikasifoglu A, Kandemir N. Clinical and laboratory parameters predicting a requirement for the reevaluation of growth hormone status during growth hormone treatment: Retesting early in the course of GH treatment. *Growth Horm IGF Res* 2017;34:31–7.
97. Cangiano B, Swee DS, Quinton R, Bonomi M. Genetics of congenital hypogonadotropic hypogonadism: peculiarities and phenotype of an oligogenic disease. *Hum Genet* 2020 [Epub ahead of print].
98. Fraietta R, Zylberstejn DS, Esteves SC. Hypogonadotropic Hypogonadism Revisited. *Clinics* 2013;68(Suppl 1):81.
99. Fredriks AM, Van Buuren S, Van Heel WJM, Dijkman-Neerincx RHM, Verloove-

1 Vanhorick SP, Wit JM. Nationwide age references for sitting height, leg length, and
2 sitting height/height ratio, and their diagnostic value for disproportionate growth
3 disorders. Arch Dis Child. 2005 Aug;90(8):807–12.
4
5
6
7
8

- 9 100. Binder G, Schweizer R, Blumenstock G, Braun R. Inhibin B plus LH vs GnRH
10 agonist test for distinguishing constitutional delay of growth and puberty from
11 isolated hypogonadotropic hypogonadism in boys. Clin Endocrinol (Oxf) 2015
12 ;82(1):100–5.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
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NOTES

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TABLES

Table I - Clinical differences between CDGP, GHD and cHH at presentation

(2) (3) (15) (16) (17) (18) (25) (26) (29) (30) (33) (44) (67) (68) (97) (99)

	CDGP	GHD	cHH
Initial Height (SDS)	>-2.5	any	>-2.5; 60% of the cases has normal height
Growth velocity	Prepubertal, concordant with bone age	Extremely reduced	Prepubertal
BMI	Reduced	Increased	Normal or increased
Bone Age (BA)	Delayed by 1-3 years	Frequently delayed; possible rare cases of normal/advanced BA for CA if sudden GHD onset occurs or precocious puberty coexists	Delayed
Predicted Adult Height (PAH)	Lower limit of TH	< TH	>TH
Past history illness	Sometimes cryptorchidism (unilateral) or phimosis	Neonatal hypoglycemia, prolonged jaundice, micropenis, midline defects	“Red flags”: cryptorchidism (bilateral), micropenis, hypospadias, renal anomalies, midline defects, hypo/anosmia
Family history	positive for CDGP in 80% of the cases	Parent's consanguinity; one family member affected if secondary to a specific mutation	One family member affected or presenting hypo/anosmia or neurologic diseases or history of CDGP

Table note:

BMI = body mass index, PAH = predicted adult height, BA = bone age, CA = chronological age, TH =target height.

Table II- Differences of five methods of adult height prediction (PAH) in terms of over-estimation or under-estimation (in cm) compared to actual final height

(27) (28) (29) (30) (69)

Method of PAH	boys	girls
Bayley and Pinneau	+3.1 cm	-0.8 cm
Roche-Weiner-Thissen (RWT)	-0.6 cm	+2.3 cm
TH	+1.7 cm	+1.2 cm
Tanner-Whitehouse Mark I (TW-MI)	-7.3 cm	-2.1 cm
Tanner-Whitehouse Mark II (TW-MII)	- 4.2 cm	-1.8 cm

Table III.— Final height (FH) in CDGP boys and girls considering their possible treatment with low dose sex steroids

(54) (55) (56) (57) (63)

	SDS FH untreated patients	SDS FH treated patients	SDS TH	ΔSDS FH -TH	SDS FH reference population
BOYS					
Crowne et al, EJP, 1991 (n = 43)	-1.6	n. r.	-0.6	-1	n. r.
Poyrazoglu et al, JPEM 2005 (n = 30)	-2.3	n. r.	n. r.	n. r.	+0.12
Couto-Silva et al, JPEM 2005 (n = 70)	-0.7	-1.2	n. r.	n. r.	n. r.
Zucchini et al, EJP 2008 (n = 29)	-1.02	-1.39	-1.12 untreated patients; -1.45 treated patients	+0.1 untreated patients; +0.43 treated patients	n. r.
GIRLS					
Crowne et al, EJP, 1991 (n = 15)	-1.5	n. r.	-0.8	-0.7	n. r.
Zucchini et al, EJP 2008 (n = 16)	-0.78	n. r.	-0.88	+0.1	n. r.

Table note: n.r. = not reported

Table IV.—*Final height (FH) in GHD boys and girls treated with rhGH at substitutive doses.*

(86) (87) (88) (89) (90)

	FH SDS	Δ SDS FH -IH	Δ SDS FH -TH
Reiter E.O. et al (n = 1258)	-0.8 (♂); -1 (♀)	n.r.	From -0.6 to +0.2
Cutfield et al (n = 369)	-1.5	+1.7	-0.5
Genentech American group (n = 121)	-0.7 $\pm$ 1.2	n.r.	n.r.
Pozzobon et al (n = 94)	-1.11	From +1.85 to +1.39	From +0.11 $\pm$ 0.80 to +0.26 $\pm$ 0.82

Table note: n.r. = not reported

TITLES OF FIGURES

Figure 1. - Genetic bases of CDGP, GHD and cHH and shared causes.

Figure 2. - Red Flags for cHH. Note that most of these features are shared with GHD and, in a minority of cases, with CDGP.

Figure legend:

ONH = Optic nerve hypoplasia

SOD = Septo optic dysplasia

HPE = holoprosencephaly

EPPG = ectopic posterior pituitary gland

PSIS = pituitary stalk interruption syndrome

MPHD = multiple pituitary hormonal deficiencies

CHARGE = Coloboma, Heart defects, Atresia of choanae, Retardation of growth, Ear abnormalities

Figure 3. —Diagnostic algorithm for the differential diagnosis between CDGP, GHD and cHH

Figure legend

- GHST = growth hormone stimulation test; Criteria for GHST : SDS H < -3 or Δ SDS TH-IH < 1.5 & GV < 1
- LDSS = low dose sex steroids; for example testosterone enanthate im 25-50 mg/monthly for boys for 6 months or oestradiol patches 12.5 µg twice weekly for girls for 6 months

**Example of priming protocol before GHST: Testosterone enanthate 100 mg im 7 days before the test for boys; E2 Valerate 1-2 mg orally (if < 20 kg or > 20 kg respectively) 3 days before the test for girls.*

*** Supportive endocrine markers (for boys):*

- Inhibin B < 35 ng/L for Tanner stage 1; < 65 ng/L for Tanner stage 2
- AMH < 20 ng/mL
- LH peak under GnRH test or GnRHα < 4-5 UI/L
- Testosterone levels < 3.6 nmol/L after 3 days or < 9.5 nmol/L after 19 days of hCG test

DIFFERENTIAL DIAGNOSIS BETWEEN CONSTITUTIONAL DELAY OF GROWTH AND PUBERTY, IDIOPATHIC GROWTH HORMONE DEFICIENCY AND CONGENITAL HYPOGONADOTROPIC HYPOGONADISM: A CLINICAL CHALLENGE FOR THE PEDIATRIC ENDOCRINOLOGIST.

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ABSTRACT

INTRODUCTION: Differential diagnosis between constitutional delay of growth and puberty (CDGP), partial growth hormone deficiency (pGHD), and congenital hypogonadotropic hypogonadism (cHH) may be difficult. All these conditions usually present with poor growth in pre- or peri-pubertal age and they may recur within one familial setting, constituting a highly variable, but somehow common, spectrum of pubertal delay.

EVIDENCE ACQUISITION: Narrative review of the most relevant English papers published between 1981 and march 2020 using the following search terms “constitutional delay of growth and puberty”, “central hypogonadism”, “priming”, “growth hormone deficiency”, “pituitary”, “pituitary Magnetic Resonance Imaging”, with a special regard to the latest scientific acquisitions.

EVIDENCE SYNTHESIS: CDGP is by far the most prevalent entity in boys and recurs within families. pGHD is a rare, often idiopathic and transient condition, where hypostaturism presents more severely. Specificity of pGHD diagnosis is increased by priming children before growth hormone stimulation test (GHST); pituitary MRI and genetic analysis are recommended to personalize future follow-up. Diagnosing cHH may be obvious when anosmia and eunuchoid proportions concomitate. However, cHH can either overlap with pGHD in forms of multiple pituitary hormone deficiencies (MPHD) or syndromic conditions either with CDGP in family pedigrees, so endocrine workup and genetic investigations are necessary. The use of growth charts, bone age, predictors of adult height, primed GHST and low dose sex steroids (LDSS) treatment are recommended.

CONCLUSIONS: Only a step-by-step diagnostic process based on appropriate endocrine and genetic markers together with LDSS treatment can help achieving the correct diagnosis and optimizing outcomes.

Key words:

puberty, short stature, central hypogonadism, growth retardation, pituitary

TEXT

Growth hormone (GH) is the most abundant hormone in the adult pituitary gland. Somatotrophs, located predominantly in the lateral wings of the anterior pituitary, represent the most abundant cell population in the pituitary (35-45% of total). GH action is crucial in mediating growth and metabolic functions in human tissues and, for this reason, is finely regulated. Neuropeptides, neurotransmitters and opiates impinge on the hypothalamus modulating the release of either GHRH, which induces GH gene transcription and hormone release, or somatostatin (also called somatotropin release-inhibiting factor, SRIF), which suppresses both basal and GHRH-stimulated GH pulse amplitude and frequency. Stimulating effects have been described from α -adrenergic pathway (norepinephrine, insulin-induced hypoglycemia, clonidine, arginine, and L-dopa, apomorphine), endorphins/enkephalins (released after physical stress and extreme exercise), from slow-wave sleep, orexigenic hormones (ghrelin and synthetic hexapeptides through growth hormone secretagogue receptor), cholinergic and serotonergic neurons, gut neuropeptides (neurotensin, VIP, motilin, cholecystokinin, glucagone), acute glucocorticoid administration and sex steroids. Inhibiting effects have instead been described from β -adrenergic pathway, anorexigenic hormones (leptine), high circulating fat free acids levels, acute hyperglycemia, hypothyroidism, chronic glucocorticoid exposure, and IGF-1, this last one as part of the hypothalamic-pituitary peripheral regulatory feedback system. The integrated interactions between these influences elicit the classical episodic GH peaks right inside a specific 24-h circadian rhythm. (1)

Gonadotropins (LH and FSH) comprise only 10-15% of the anterior pituitary cell population but their role is crucial for gonadal stimulation and reproduction. Hypothalamic GnRH pulse amplitude and frequency determines the physiologic pattern of LH and FSH secretion. Kisspeptin neurons undergo a dynamic process of prenatal-postnatal maturation that enables them to establish connections to GnRH neurons early in development. KNDy neurons located in the arcuate nucleus of the hypothalamus and co-expressing Kisspeptin, neurokinin B and dynorphin represent the GnRH pulse

generator in both sexes, which then in turn stimulate gonadotroph cells in the pituitary, triggering puberty induction and progression. After a first period of action in utero and in the first months of life (the so-called mini-puberty), this axis is switched off during childhood and reawakens itself in the peripubertal period. Many neurotransmitters from the limbic system, the brain stem and from other hypothalamic nuclei stimulate (glutamate, norepinephrine, kisspeptin) or inhibit (GABA, endogenous opioid, dynorphin) the hypothalamus-pituitary-gonadal (HPG) axis. Other influences may derive from glial cells, periferic steroids milieu and gonadic peripheric production of activin/inhibin. Leptin, a product of peripheral adipose tissue, is also a positive regulator of the hypothalamic-pituitary-gonadal axis, signaling energy availability to the central nervous system. (2)

(3)

Gonadal steroids then reciprocally have a wide influence on GH secretion and action. Testosterone stimulates central GH secretion mainly through its aromatization to estrogen: in fact, anti-estrogen tamoxifen is able to significantly reduce in normal, and in hypogonadal men on testosterone replacement therapy, the 24-h mean serum GH concentrations, the mean GH pulse amplitudes, and the serum IGF-1 levels. (4) As further proof, the administration of a non-aromatizable androgen (dihydrotestosterone) is not able to elicit GH secretion. (5) Testosterone additionally acts peripherally, amplifying GH-mediated secretion of IGF-1, sodium retention, substrate metabolism and protein anabolism. Exogenous estrogens are instead able to stimulate GH secretion when given orally, due to their first-pass hepatic inhibition of IGF-1 production. This effect can be avoided using transdermal routes, at least if physiological doses of estrogens are given. (6)

Instead it is still unresolved whether, in females pituitaries, estrogens have a central direct stimulatory role on somatotrophs, since stimulated GH secretion proved to be similar between women with premature ovarian failure (either assuming or not hormonal replacement therapy) and aged-matched controls with normal ovarian cycles. (7)

Animal models studies proved the importance of the sex steroids impingement on fetal pituitary somato-mammotrophs cells store, which will subsequently determine the GH reserve in childhood

and adulthood. (8) (9) Similarly, human studies have also demonstrated that, physiologically, during puberty the activation of the hypothalamic-pituitary-gonadal axis leads to a large increase in the circulating sex steroids concentration, which then enhance the pulse amplitude of GH secretion, increase IGF-I concentrations and the anterior pituitary size. (5) (10)

Many association studies in normal children have also proved a physiological threefold increase in GH secretion following the increase in gonadal steroid concentrations during puberty. (11)

GH secretion has also proved to be reduced in hypogonadal children (12) but increased in precocious puberty, whose successful therapy is able to normalize GH secretion. (13)

Because of these multiple interconnections between GH and HPG axis, it is not infrequent that a child in peripubertal age may present to a pediatric endocrinologist with short stature and/or reduced growth velocity with associated delayed puberty. (**Table I**). For the same reasons, subnormal GH peaks to physiological or pharmacological stimuli are frequently found in normal prepubertal children, but they have proven to fully normalize when they will achieve stages 4 or 5 of Tanner maturation or after estrogen administration. (14)

While constitutional delay of growth and puberty (CDGP) may be the most probable diagnosis in a child presenting with short stature and pubertal delay, especially for males, growth hormone deficiency and congenital hypogonadotropic hypogonadism must at the same time be ruled out. Otherwise, in children with true GHD or cHH left untreated, adult height and pubertal maturation will lack to fully develop normally, resulting in important psycho-social discomfort as well as long term medical sequelae in these children (permanent short stature in GHD, cryptorchidism and risk of testicular cancers/lack of fertility in cHH, osteoporosis and alteration of body composition in both conditions). As growth is a good indicator of a child's health, the increasing attention on children wellness and well-being especially in the more developed countries has been a matter of medical attention over years. Moreover, as the child grows into adulthood, fertility potential becomes an important issue, which is also a major concern for parents.

EVIDENCE ACQUISITION

1. Constitutional delay of growth and puberty (CDGP)

1.A Definition

The term CDGP describes children who have a normal variant of maturation tempo characterized by short stature with relatively normal growth rate during infancy, but showing deceleration during peripubertal period possibly due to the lack of growth spurt physiologically driven by the sex hormones rise. According to the pubertal status, 3 different “categories” of CDGP children may be defined:

- CDGP type 1: Prepubertal children (Tanner stage G1 for boys ≥ 14 yo or B1 for girls ≥ 13 yo), as reported by the 2007 ESPE classification, (15) usually associated by slow growth rate, delayed bone age (delay of 1 year or more compared to chronological age) and a positive family history of delayed puberty
- CDGP type 2: Pubertal children but displaying slow or stuttering progression of sexual characteristics, with a genital Tanner stage less than – 2 SDS compared to normal age-matched peers, defined by the use of puberty nomograms (16)
- Possible CDGP type 3, according to Stalman et al: if before 14 (for boys) or 13 (for girls) years old if, during his/her growth, three out of four conditions are met: i) Tanner stage < -1 SDS defined with the use of puberty nomograms; ii) bone age delay > 1 year compared to chronological age; iii) being short for target height (TH); and iv) positive family history of pubertal delay (menarche in the mother > 15 yo; late paternal growth spurt and shaving). (17)

1.B Prevalence

CDGP is a relative common condition, affecting 2-2.5% of the population. (18) This alteration is extremely common in boys, and less in girls. It is estimated that up to 80% of boys and around 33% of girls displaying delayed puberty have a self-limited condition. (19) However, prevalence in CDGP females ranges widely : in a large Georgian cohort of female adolescents with delayed puberty, for example, the prevalence of CDGP in girls is accounted to be lower and around 10%, against the great majority of the female cohort displaying organic diseases (premature ovarian failure/absence of uterus or vagina). (20) Because in family series reports males and females are described to be equally affected (19), a gender selection bias seeking medical attention has been postulated. (21)

1.C Clinical presentation

Children displaying CDGP may frequently seek for medical attention for short stature, which is usually not severe (> -2.5 SDS) and for reduced growth velocity (< -2 SDS) together with a prepubertal stage of sex maturation. Although growth velocity (GV) may be reduced compared to normal pubertal children, it is frequently found to be > 3.6 cm/year in boys. On the other hand, GV seems not discriminative at best for different etiologies of delayed puberty in girls. (22)

Because CDGP patients are sometimes poor eaters (23) and need higher caloric intake compared to age-matched controls (24), BMI is often found to be at lower range, which is an important concern for parents and contributes possibly independently to the reduced growth. Of interest, nearly 25% of boys with CDGP come with the diagnosis of attention deficit with hyperactivity disorder (24). Even though adrenarche/pubarche derive from adrenal rather than HPG axis activation, delayed puberty in these patients consists of a delayed gonadarche together with absence of pubarche/adrenarche. (25)

Looking at growth charts may be useful. CDGP boys are born with normal length, have a decreased growth pattern compared to target percentile until 2 years old, then tend to level till 5 years old,

starting from when a height SDS loss is already detectable, reaching its maximum at pubertal age.
(26)

For a complete auxological workup, a systematic assessment of bone age is warranted, either using Greulich-Pyle or Tanner Whitehouse methods (referring to its second edition of 1983 TW2, or its third revised edition of 2001 TW3) or informatic programs that are rising into attention for the more accurate bone age assessment in non-american non-caucasian ethnicities. (27) In CDGP subjects bone age is frequently found to be delayed by at least 2 years compared to chronological age, even though it can range from 1 to 3 years less, so that if we bring the child back on his biological age the growth percentile may be adequate for the family target. (28)

The prediction of adult height based on bone age ($PAH =$ predicted adult height) may also be very useful because it frequently suggest that a child with CDGP will lay within the lower range of his/her target height (29). To this regard it is worth explaining that different methods for final height prediction based on bone age exist, and according to the method used there may be some overestimations or undestimations of the predicted final height. (**Table II**) In the matter of question, the most used Bayley and Pinneaud system has been proved to overestimate boy's final height, raising doubts of the possibility of truly rely on this method for adult height prediction. (29) For this reasons, new models of bone age prediction in CDGP patients have been recently established. (30)

Clinical history and examination of the child should point out the so called "red flags" i.e. cues orienting the clinician towards specific diagnostic subgroups (**Fig. 2**). To this extent it is worth noticing that in CDGP patients cryptorchidism may be found in a low percentage of cases (2%) mostly as a unilateral finding. (18) Up to 80% of children diagnosed as CDGPs have at least one family member in which self-limited delayed puberty has occurred, which can be also taken as an important diagnostic tool; however, a family history of CDGP does not exclude cHH given the incomplete penetrance and variable expressivity of cHH phenotype within pedigrees (of which delayed puberty,

subfertility and hypo-anosmia can also be enriched). (2) (3) A new observation of our group introduced into this complex clinical picture also TBX3 mutations accounting for Ulnar Mammary Syndrome (UMS), and several reports of UMS families describe the variable association of either CDGP, more frequently, either of true cHH defects and GHD, in a minority of cases (31). **(Figure 2)**

1.D Endocrine workup and the role of sex steroid priming before growth hormone stimulation tests

Priming refers to the administration of sex steroids to prepubertal or early pubertal children prior to GHST. The evidence to support this practice is low, however suggested by the 2016 International Guidelines for growth hormone deficiency and idiopathic short stature. According to these guidelines, priming is suggested for prepubertal boys older than 11 or prepubertal girls older than 10 years with adult height prognosis within -2 SD of the reference population mean in order to prevent unnecessary rhGH treatment of children with a more probable diagnosis of constitutional delay of growth and puberty. (32) (33)

The studies supporting this approach are however of limited evidence, considering the small group of patients of the studies analyzed, yet sometimes conflicting. Marin et al showed a 95% rate of normalization in 11 normal children after administration of ethinylestradiol (EE) $40 \mu\text{g}/\text{m}^2$ in 3 divided doses with meals for 2 days and, compared to placebo, a rise in both GH peak from 7.6 ± 4.4 on placebo to $18.7 \pm 9.2 \mu\text{g}/\text{L}$ on EE and of the lower limit of the 95° confidence interval from 1.7 to $7.2 \mu\text{g}/\text{L}$. (14) Martinez et al, similarly, showed in a randomized control study a significant increase in GH peaks under a sequential arginine-clonidine test when 44 short normal children were primed with estradiol valerate (E2) orally (1 or 2 mg depending on the weight $<$ or $>$ of 20 kg, respectively) in three consecutive days before testing, compared to placebo: in these children GH mean peak

increased from 17.8 ± 10.9 on placebo to 27.9 ± 14.5 $\mu\text{g/L}$ on E2, increasing diagnostic accuracy of GHST from 90% to 95%. (34) Analogously, Molina et al proved that 39 children primed with either 100 mg i.m. of testosterone enanthate for 5-8 days (in boys) or with 1 mg estradiol valerate daily for 3 days (in girls) were able to significantly increase GH mean peaks under clonidine stimulation test, either if diagnosed as CDGP (from 5.83 ± 2.67 to 17.42 ± 8.46 $\mu\text{g/L}$) or as GHD (from 3.68 ± 2.34 to 5.95 ± 2.76 $\mu\text{g/L}$). (35) Muller et al showed a normalization (GH peak > 10 $\mu\text{g/L}$) after arginine test in 77% of 26 peripubertal boys primed with 100 mg of testosterone enanthate 3-10 days before the stimulation. (36) Taken together, these studies led to the suggestion that estrogen priming may reduce the number of false positive children (i.e. false non responders to a GHST) avoiding unnecessary rhGH treatment in a high proportion of children under assessment for short stature.

Other studies have however shown opposite results. Soliman et al proved that in children above 9 years of age under investigation for short stature with a clonidine stimulation test, the proportion of patients displaying GH peaks > 10 $\mu\text{g/L}$ did not differ between the primed group (pre-treated with 25 mg of intramuscular testosterone depot 7-10 days before the test for boys or with 1,25 mg of oral conjugated estrogens for 3 days before the test for girls) compared to the unprimed group (52% vs 47% respectively, N.S). (37) Most importantly, only one follow-up study on 50 boys was able to prove that avoiding treatment in children with subnormal unprimed but normal primed GH peaks under GHST does not result in an impaired final height compared to their midparental target (-1.27 vs -1.38 SDS, respectively). (38)

Larger follow-up studies considering both males and females and looking at final height of children undergoing primed GHST are lacking. However, taken together, these studies evoke the idea that priming before GHST may reduce the false positive diagnosis of growth hormone deficiency, avoiding unnecessary rhGH treatment in many children under investigation for short stature. It is however estimated that only 30-40% of pediatric endocrinologists prime peripubertal children prior to GH stimulation testing in routine clinical practice. The limited diagnostic application is justified

by some experts based on considerations of priming as an artificial stimulus generating false transient normal GH peaks under GHST. (39)

Beyond GHST, no endocrine marker can be fully reliable in the diagnosis of CDGP. Nevertheless, in males, measurable levels of Inhibin B (> 35 ng/mL) have been proposed to fairly correlate with the maturation of Sertoli cells in the gonads, hence indirectly proving that, in the first months of life, mini-puberty has occurred, pointing out a CDGP rather than cHH diagnosis. (40) AMH has also proved to be slightly higher in prepubertal CDGP compared to cHH, however not discriminative for peripubertal CDGP (Tanner stage 2) where it is found to be lower, thus not giving advantages compared to inhibin B alone. (40) (41) In prepubertal CDGP the combination of Inhibin B at a cut-off ≥ 28.5 ng/L with AMH at a cut-off of ≥ 20 ng/mL has however proved to increase the specificity of CDGP diagnosis up to 83%. (41) Other markers of testicular (INSL3), and adrenal (DHEAS) activity have not proved to be discriminative for the differential diagnosis between CDGP and cHH. (41)

The restriction of most of the studies to the male cohorts does not allow to use these endocrine markers for girls. At the same time, in females Inhibin B levels are less useful as they are lower and a less significant rise is seen at puberty. (42)

Basal gonadotropin levels are not discriminative as they are supposed to be low either in prepubertal CDGP or in cHH cohorts. If in CDGP children puberty is in a very initial stage, basal gonadotropins levels may be found to be more than 0.2 UI/L in both genders with the use of immunochemiluminometric assays, which could be a simple and useful marker against a severe cHH form. (43) Moreover, HPG axis stimulation upon GnRH test or following a GnRH agonist (GnRHa) has also been proposed (22) (42) (43), however up to 30% of children diagnosed as either CDGP or cHH overlap their LH peaks under stimulation. (44) Despite some promises for longer GnRH test protocols (up to 36 hours), these tests have suggested to be helpful only in a few studies but without

high diagnostic accuracy. Similarly, the use of GnRHa (Naferelin, Leuprolide, Triptorelin, Buserelin) has proved to elicit higher LH peaks but the low sample size of these studies, the presence of overlapping results with cHH cohorts and the absence of a clear diagnostic threshold limit their diagnostic utility. (44)

HCG test has also been postulated as a useful diagnostic tool for the differential diagnosis between CDGP and cHH (see below section 3.D) (45) (46).

Kisspeptine boluses have also proved to heterogeneously increase LH responses in CDGP patients (47) in research settings, but this approach is not yet routinely performed in clinical practice.

Another important tool that can be taken into account to differentiate CDGP from other cohorts is the evidence of a self progressing pubertal development after a 3-6 months treatment trial with low dose sex steroids (LDSS). (3) (48) After LDSS withdrawal, an Inhibin B > 94.7 ng/mL and LH peak after GnRHa > 14.7 U/L have been also proven to discriminate at best CDGP upon cHH. (49)

1.E GENETIC TESTS

Genetic basis of CDGP are supposed to be different from those of cHH. Even though, heterozygous variants in cHH putative genes have been found in up to 7% of the CDGP cohorts (18), the current scientific literature tends to consider the hereditary basis of CDGP as a different, yet mainly still unknown, genetic field. Observational studies have demonstrated that several different inheritance patterns can occur in CDGP, including autosomal dominant pattern (in the majority of the cases, with or without complete penetrance), autosomal recessive, bilinear (both parents affected) or X-linked. De novo mutations can also occur. (19)

Four genes clusters have been demonstrated in CDGP cohorts by Howard et al. with exome sequencing in Finnish CDGP index patients: (3)

- 1) Fat Mass and Obesity-associated (FTO) gene (in three of 67 families)
- 2) Heparan Sulfate 6-O-Sulfotransferase 1 (HS6ST1) gene (in one in 67 families)
- 3) Enhanced At Puberty 1 (EAP1) gene (in two in 67 families)
- 4) IGSF10 rare sequence variants in up to 16.4% of the CDGP cohorts, a gene which encodes a protein that belongs to the immunoglobulin superfamily. However, the role of IGFS10 in delayed puberty remains open, as in a follow-up study, IGSF10 mutations were not reported to be enriched in CDGP patients when compared to controls.

Mutations in the pituitary transcription factor POUF1 (PIT1) involved in pituitary thyrotrophs and lacto-somatotrophs cells development and hormonal expression may present with pubertal delay or with absent or delayed adrenarche, resembling delayed puberty (50). Mutations in GHSR, a growth hormone secretagogue receptor gene whose endogenous ligand ghrelin produced from gastric cells increases GH pituitary secretion, have been found to be mutated in both GHD and CDGP patients. (51)

Some known syndromic conditions may present with either CDGP, GHD or cHH within family pedigrees (Prader Willy Syndrome or CHARGE syndrome). Moreover, as already mentioned, TBX3 mutations accounting for Ulnar Mammary Syndrome (UMS) have been described in literature also linked to all of these scenarios including CDGP in many family pedigrees. (31).

The genetic basis of CDGP and shared causes with GHD and cHH are illustrated in **Figure 1**.

1. F FINAL OUTCOMES AND THERAPEUTIC OPTIONS

Given that CDGP is a self-limiting condition, reassurance and watchful waiting are reasonable approaches to treatment. On the other hand, however, new evidences towards long-term sequelae

associated with delayed puberty are rising into clinician's attention, from the possibility of early menopause and low bone density to a worse psychosocial well-being and peer's relationships (19). Moreover, association studies investigating earlier or later timing in puberty in both genders have been linked with a wide range of adverse health outcomes, including cancers, cardiometabolic, gastrointestinal, musculoskeletal and neurological pathologies. (52)

Additionally, because in CDGPs the magnitude of the growth spurt may be blunted as the child gets older, not infrequently these children will not be able to reach their full growth potential. (53) To this regard, final height in CDGP patients has been widely investigated in boys and many studies have proven that untreated CDGP patients are prone to become shorter either compared to their mid parental targets and/or their peers displaying normal timing of puberty, whereas if a treatment with LDSS is given to the child for a short period a time, FH has proved to become similar or better compared to those left untreated. (54) (55) (56) (57) (**Table III**) This effect may expire if higher doses of testosterone are used. (58) Testosterone therapy (in the form of testosterone esters/undecanoate, given either intramuscularly, transdermal gel, or orally) in this group of patients appears a safe and well tolerated approach that can favor a prompt recovery of hypothalamus-pituitary axis after discontinuation of treatment if low doses are given. (59) The treatment schedules are quite variable, and the efficacy of the treatment is clearly an under-investigated area in adolescent medicine with very few randomised trials available. Letrozole treatment has also been proven to give benefits in CDGP boys compared to testosterone in terms of pubertal progression, even by eliciting different HPG axis responses (increasing gonadotropins and Inhibin B, leading to larger testis size). (22) Both therapies (testosterone 1mg/kg/month or letrozole 2.5 mg/day) had anyway proven to elicit changes in Sertoli cells markers (i.e. decrease in AMH), reassuring about safety in developing mature seminiferous epithelium with both approaches. (60) Other therapies such as oxandrolone have also been suggested for treatment of CDGP, but long term data are lacking. (61) (62)

Very few follow-up studies are instead available for CDGP girls, and data on treatment with low dose estradiol are almost lacking. (57) (63) (**Table III**) This is possibly due to the lower rate of presentation to medical attention of girls with pubertal delay compared to boys (26). For girls, natural and transdermal estradiol preparations are preferred.

A reasonable therapeutic approach for CDGP children may be made either with 50 mg testosterone enanthate intramuscularly monthly for 3-6 months either with 10 mg testosterone gel 2% daily for 3 months for boys, or with ¼ of a 25 µg patch of 17-β estradiol once or twice weekly for 6 months for girls. (3) (64) (65)

Taken together we believe that CDGPs should not be considered as a para-physiological variant of maturation-tempo but, perhaps more appropriately, as a condition characterized by a significant delay of pubertal timing needing treatment with LDSS for a short period of time in order to normalize their growth pattern and achieve full growth potential and limit psychosocial and medical issues associated with delayed puberty.

2. Idiopathic growth hormone deficiency

2.A DEFINITION:

Growth hormone deficiency is a complex yet rare diagnosis in a child, that should be based on the whole thing of auxological, anatomic and laboratory's findings. (33) This condition can be challenging to differentiate from CDGP as in both settings there is a height SDS deflection and relatively low height velocity compared with cross-sectional population references. Moreover, GHD may also overlap with cHH clinically, especially when multiple pituitary hormone deficiencies (MPHD) or shared genetic mutations with the condition occurs.

2.B PREVALENCE

The prevalence of GHD is around 1 child every 4.000 - 10.000. It is therefore a rare condition and mainly a diagnosis of exclusion upon other possible causes of poor growth (malnutrition, chronic liver and renal diseases, pulmonary diseases, syndromic conditions, iron overload, hypothyroidism, diabetes, skeletal dysplasias, inborn errors of metabolism, and CDGP).

Prevalence of GHD worldwide may ranges widely due to the lack of standard diagnostic criterias (for example, it has been overestimated as up as 1:1800 in Sri Lanka probably due to more liberal diagnostic standards) and also due to referral bias. Even though there are no apparent racial differences in the incidence of GHD, The National Cooperative Growth Study (NCGS), Genentech's study on North American database, revealed that 85% of patients receiving GH treatment for idiopathic GHD were white, 6% were black, and 2% were Asian and similar distributions were seen with patients with other forms of short stature. Additionally, patients with GHD from organic causes such as tumors and radiation, may be much more prevalent in more industrialized countries, where medical intervention on population is deepened. (66).

2.C CLINICAL PRESENTATION

Children with GHD may display severe armonic short stature, a reduced height compared to the target height (TH) and/or a poor growth rate. The past medical history may help the clinician if neonatal hypoglycemias, midline defects, prolonged jaundice or MPHDs are present. (67)

In growth charts of boys with congenital GHD a continuous decrease in height SDS from birth on occurs without any levelling (68). Moreover, an increase in fat mass has been described in patients left untreated giving them a "cherubic appearance", which can also be taken as an important diagnostic tool against a presumptive CDGP diagnosis, where BMI is classically found to be low.

Bone age, either assessed with Greulich & Pyle or Tanner-Whitehouse methods, is again found to be delayed due to the low GH-IGF-1 levels modelling bone maturation. However, there may be three possible confounding scenarios in which bone age may not be delayed: (i) recently acquired GHD, (ii) obesity, which is typically associated with advanced bone age, and (iii) concomitant precocious puberty, a scenario where sex hormones can trigger bone maturation. (33)

PAH based on bone age will clearly show an adult height well below the genetic potential of the child. As already mentioned, many methods for PAH predictions exist (**Table II**): target height, which is the most used method of height prediction in clinical practice, has anyway been associated with a higher prediction error compared to the less used Roche-Weiner-Thissen method (RWT) in a cohort of severe GHD. (69) Therefore, this method might be superior for the prediction of adult height in a child with short stature. It is however clear that CDGP can come into differential diagnosis mainly with cases of recently acquired GHD or, more frequently, partial idiopathic GHD (pGHD).

2.D ENDOCRINE WORKUP, role of sex steroid priming before GHST and pituitary MRI

Serum IGF-1 levels are frequently low in GHD compared to pubertal aged-matched peers and its determination in peripubertal children should be assessed within adjusted reference ranges for actual pubertal status. (70) An IGF-1 level > 0 SDS for gender and age specific range makes the GHD diagnosis highly unlikely (71) and it can be used as a screening tool for GHD. However, when IGF-1 is found to be low, a series of other conditions need to be excluded (such as malnutrition, chronic diseases, hepatic diseases).

However, IGF-1 levels may be influenced by a series of other conditions, therefore an evaluation of GH reserve in the majority of cases is warranted.

Regarding growth hormone stimulation tests, in the absence of a “gold standard”, it is impossible to precisely define the sensitivity and the specificity of any test for GHD. Insulin tolerance test (ITT) may be considered the gold standard for the evaluation of GH reserve, but its intrinsic risks hamper the definition of normal responses thresholds. Some information in specificity can be learned by comparing the results obtained in normal children. It is well known that within normal children and/or children with idiopathic short stature 10 to 25% will show a GH peak $< 7 \mu\text{g/L}$ and up to 23-49% will have a peak $< 10 \mu\text{g/L}$. (72) This may suggest that every individual stimulus may incorrectly classify almost half of the children as false positive GHD, if higher cut-offs are assumed. To this regard, an attempt of progressively reducing the diagnostic cut-off of GH peak by $7 \mu\text{g/L}$ has been recently proposed by the latest Growth Hormone Research Society Guidelines (33), although the previous cut-off (at 10 mcg/dL) has not been revised yet. Also in Italy, the cut-off of 8 mcg/dL has just been confirmed in the latest revision of nota AIFA 39.

Sensitivity of GHST, can hence be deduced by comparing one positive test to the positive response of another investigation, for example correlating low IGF-1 concentrations to the failed results on GHST. However sometimes the tests can be discrepant and in this scenario it is difficult to determine whether this is due to the poor sensitivity of the IGF-1 test or to the poor specificity of the GHSTs themselves.

To increase GHST specificity, sex steroids priming preparation should be performed before testing. However, in contrast with CDGP cohorts, sex steroid priming before growth hormone stimulation testing has been associated with conflicting benefits in the GHD cohorts in literature. Even though in the already cited study of Molina primed GHD children were able to significantly increase GH mean peaks under clonidine stimulation test from 3.68 ± 2.34 to $5.95 \pm 2.76 \mu\text{g/L}$. (35), in the study performed by Martinez et al, GHD children did not significantly increase GH mean peaks under sequential arginine-clonidine test after estrogen priming (GH peak 3.1 ± 2.4 on placebo and 4.5 ± 2.7

1 $\mu\text{g/L}$ on E2, NS), suggesting that the effect of priming is irrelevant in those patients with more severe
2
3 forms of GHD, thus displaying lower GH peaks under stimuli. (34)
4

5
6 Because of the poor specificity and the low reproducibility of the GHST, the cardinal tool for a correct
7
8 diagnosis is again a pathological short stature and/or growth deceleration (i.e. height < -2 SDS,
9
10 difference from target height < -1.5 SDS, growth velocity < -2 SDS) highlighted by clinical
11
12 examination, considered to be the undeniable starting point. Pathological auxological criterias are in
13
14 fact thought to increase the pre-test probability of GHD in children under investigation, especially in
15
16 those without any other risk factor (i.e. pathology including ectopic posterior pituitary and/or
17
18 abnormality of the pituitary stalk, irradiation, at least other pituitary hormone deficiency or carrying
19
20 a pathogenic genetic mutation), and in these patients two abnormal tests are frequently needed before
21
22 GHD is diagnosed. (32) Instead, in a patient with pathologic auxological criteria, a normal GH peak
23
24 in one test rules out GHD in most of the cases. However, as in some conditions (cranial irradiation,
25
26 optic nerve hypoplasia, traumatic brain injury, or known genetic conditions) GHD may evolve over
27
28 time, a second GHST is frequently suggested at a later time point. (33)
29
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31
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33 A Magnetic Resonance Imaging (MRI) of the hypothalamus and pituitary glands should be performed
34
35 in all patients diagnosed with GHD in order to rule out anatomical defects of the hypothalamic-
36
37 pituitary region or tumoral brain lesions. This is important for predicting the likelihood of other
38
39 pituitary deficiencies, the utility of genetic testing and the probability of persistent GHD after
40
41 attainment of final height. (33) In around 15% of cases an anatomic malformation of the
42
43 hypothalamus-pituitary axis is found, but in the majority of the children, no cause can be identified
44
45 and this group of patients are often referred as having idiopathic growth hormone deficiency (IGHD).
46
47 (73) These children diagnosed as IGHD frequently carry transient forms of growth hormone
48
49 deficiency during childhood and the rate of normal retesting has been found to be as high as 80% in
50
51 many papers (see later). (74) (75) (76) (77)
52
53
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55

Major anatomical abnormalities (including thin or interrupted hypophyseal stalk, suprasellar or intrasellar tumors invading the hypothalamus-pituitary anatomy) are known risk factors for multiple pituitary hormonal deficiencies (MPHDs) and of persistent GHD in adulthood. (74) (75) (78) Ectopic posterior pituitary with an otherwise normal anterior pituitary gland is also considered to increase by 26% the probability of adult persistent GHD (79). A small anterior pituitary, defined as a gland < -2 SDS of normal size using the few available standards for normal population categorized for age and gender (80) (81) (82), may incidentally be found on MRI. A hypoplastic anterior pituitary gland by itself is not sufficient to diagnose GHD, but may indicate the need for a more extensive evaluation of the anterior pituitary function. (33) Pituitary size should be interpreted in the context of pubertal status. It is in fact well established in normal subjects that the pituitary gland increases its size during puberty (a phenomenon even more pronounced in females), reaching a plateau at approximately 20 years old, and a physiologic decline thereafter. (83)

Many Authors studying pituitary MRI of IGHD children have indeed found a significantly low volume of the pituitary gland compared to age-matched peers, speculating that there could be an association between anatomic and functional abnormalities, meaning that those patients with smaller pituitary glands may have a smaller somatotrophs volume thus secreting less growth hormone. (84) (85)

2.E GENETIC TESTS

The majority of patients with growth hormone deficiency is considered of idiopathic origin. Some isolated growth hormone deficiency are secondary to a genetic mutation within the GH gene locus named GH1 (IGHD type IA or IB) or in its introns (IGHD type II), or may be transmitted as an X-linked trait together with hypogammaglobulinemia (IGHD type III) (73).

An exome or a genome wide approach is nowadays thought to be the best diagnostic option, yet sometimes not available in clinical settings. A panel-oriented gene analysis for the most frequent

genes associated with GHD could be a reasonable approach. Some features are associated with a high pre-test probability of a genetic mutation: severe short stature at presentation (< -3 SDS from population or from target height), multiple pituitary hormonal deficiencies, anatomic alteration of the CNS, familial segregation with an autosomal dominant or recessive pattern or with a history of consanguinity. (33) Genes associated with GHD and shared with CDGP and cHH are illustrated in **Figure 2**.

2.F FINAL OUTCOMES AND THERAPEUTIC OPTIONS

Even though growth hormone therapy has been given since 1958, starting with extracted GH from human pituitary glands, and then from 1980s with the use of the recombinant human GH (rhGH) technology, little is still known about its effect on adult height and conflicting data exist on long-term studies, with some showing failure to reach the genetic mid-parental target height but others, by contrary, showing successful target height achievements. It is clear from reports that individual height response may vary considerably even with individualized treatment regimens, mainly depending on the chronological age at the start of therapy, on the severity of the GH deficiency after stimulation tests and on the stage of pubertal development. (86) (87) (88) (89) (90) (**Table IV**)

In fact, as a result of the lack of a uniformed cut-off limit for GHD, of the heterogeneity of the GHST used, and the fact that sex steroid priming is frequently not performed, it is not infrequent that a lot of these studies on IGHD children erroneously include also CDGP patients, who may present with short stature and pubertal delay. For this reason, outcomes reported in papers on final height of patients receiving rhGH treatment may vary widely and in some cases an overlap in terms of FH outcomes is seen with CDGP cohorts, especially for CDGP girls or for CDGP boys treated with LDSS. (**Tables III and IV**) If very low growth outcomes are achieved after treatment with rhGH (SDS FH-IH around +1.2 only), possible other causes of short stature should be considered. (91)

1 Roughly, a standard substitutive dose of rhGH should be 25-35 $\mu\text{g/kg/die}$ but the dose of rhGH should
2
3 then be individualized according to GH responsiveness aiming for the lowest dose able to induce a
4
5 response in height velocity, possibly using prediction models. (92)
6

7
8 Retesting GH status at end of growth is currently recommended by the European Society for Pediatric
9
10 Endocrinology guidelines for all patients with GHD except for those children with multiple (≥ 3)
11
12 pituitary hormone deficiencies regardless of the etiology, for GHD with a documented causal genetic
13
14 mutation or specific pituitary/ hypothalamic structural defect. (32) To this extent, small pituitary
15
16 glands are not considered as anatomic defects, since a high proportion of normalization at retesting
17
18 in children with this condition has been demonstrated (74)
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20
21 Guidelines differ on suggested cut-offs for GHD diagnosis during transition under ITT test, with
22
23 American guidelines using lower cut-offs (5 $\mu\text{g/L}$) (93), European Society for Pediatric
24
25 Endocrinology guidelines suggesting a cut-off of 5.6 $\mu\text{g/L}$ (94) for immunometric assays, and GH
26
27 Research Society guidelines suggesting a cut-off of 6 mg/L . (33)
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29

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31 Studies using standard cut-offs have confirmed that around two thirds of patients diagnosed as GHD
32
33 during childhood-puberty, proved to have transient form of GHD. Tauber et al reported a 67% rate of
34
35 normalization at retesting in IGHD patients, which was increased up to 71% in children previously
36
37 diagnosed as partial GHD (GH peak at diagnosis between 5-10 $\mu\text{g/L}$). (76) Moreover, a proportion of
38
39 normalization of GH secretion in adulthood has been demonstrated as high as 87% for children with
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41 isolated IGHD of childhood onset (75).
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45 Additionally, early retesting of children diagnosed as idiopathic GHD with a normal MRI has turned
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47 out to normalize in up to 85% in 1-6 months after diagnosis (95), and has been recommended. (96)
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51 The reasons for the high proportion of patients who are apparently increasing their GH secretion at
52
53 retesting are however likely to be multifactorial. Long-term exposure to sex steroids may increase
54
55

pituitary size and GH secretion, some children may have had a form of transient GHD and, eventually, some others may have been incorrectly diagnosed as GHD, given the problems associated with biochemical testing outlined previously.

3. Congenital Hypogonadotropic Hypogonadism

3.A DEFINITION

Congenital Hypogonadotropic Hypogonadism (cHH) is an organic and heterogeneous disease due to abnormal secretion or action of GnRH. It may be characteristically associated with an olfactory defect such as anosmia/hyposmia, the so called Kallmann syndrome (KS), or may occur in the context of normal olfaction, also known as normosmic HH (nHH). (2) The possible association with olfactory defects is related to the common embryonic origins of the GnHR-secreting and olfactory neurons, which both migrate from the olfactory placode to their final location in the brain through the cribriform plate of the ethmoid bone.

cHH is defined by the criteria of a prepubertal state at the age of >14 years and by a testicular volume not reaching a value of ≥ 5 mL during a 24-month follow-up despite a short trial with testosterone. A final differential diagnosis between CDGP is made if spontaneous puberty does not occur by the age of 18 years old. (44) However, the wait-and see approach is clearly not recommended because of the known psychosocial and medical issues associated with a pubertal delay already cited above.

3.B PREVALENCE

cHH is a rare disease with a frequency of 1:4000-6000 males with a 3-5:1 male:female sex ratio in tertiary centers. (97) The prevalence worldwide is difficult to determin, but it has been reported

spanning from 1:10.000 to around 1: 86.0000, but when interpreting these findings methodological limitations of case ascertainment by medical record review should be kept in mind. (98) cHH may be familial or sporadic.

3.C CLINICAL PRESENTATION

A wide spectrum of phenotypes have been described.

- Complete cHH starting form foetal/neonatal life
- Complete cHH starting from the peripubertal period
- Partial cHH with an initial spontaneous drive of pubertal development but a subsequent sudden arrest (one third of the cases)
- Same as above with a reversal of GnRH secretion during adulthood (almost 10% of the cases)
- Adult onset cHH

The phenotype may vary accordingly. In boys the presence of micropallus, mild hypospadias and/or (bilateral) cryptorchidism may indicate a complete defect of foetal/neonatal origin, whereas the absence of these signs but the lack of puberty to start or to progress till Tanner 5 may indicate either a complete peripuberatal form or a partial form of cHH, respectively, which may be difficult to differentiate from self-limiting constitutional delay. In females the scenario is even less well-defined, since the foetal/neonatal phenotype of cHH females is still unknown and possibly indistinguishable from other phenotypes. Clinical features of cHH of peripubertal onset or of partial forms in females are again the absence/ the incomplete self progressing puberty and primary amenorrhea, both overlapping with CDGP females, and the presence of eunuchoid proportions. (97) Interestingly, in both sexes, gonadarche alone is found to be absent, whereas adrenarche and pubarche may be present. (25)

It is therefore clear that a complete physical examination considering limbs' asymmetry (ratio arm span/standing height > 1, arm span > 6 cm of standing height and for eunuchoid proportions, sitting

height/leg length ratio $< 0.8-1$) may orient clinicians towards cHH when disproportionateness is clinically detectable, taking available clinical chart for normal children as a reference. (99)

Short stature at pubertal age in cHH accounts for 40% of the cases, further rising complexity to the picture, however, an accurate analysis of the patient's growth chart may help in the diagnosis. In boys with cHH, in fact, height SDS does not differ to that of target potential till the first 5 years of life but a typical decrease at puberty occurs, with an inverse correlation with BMI, which is frequently found to be in the higher part of normal range. (30) Similar data on growth charts are however lacking in literature for cHH girls.

Apart from these specific endocrine phenotypes, other specific features, both neurological and extra-neurological, may be present. Associated neurological phenotypes, include a defective smell sensation, sensorineural hearing loss, color blindness (daltonism), nystagmus and bimanual synkinesis (mirror movements). Hypo-Anosmia, that can be found in 50% of the patients, is also a pathognomonic sign of Kallman syndrome and may help in the diagnostic process. (2) The olfactory competence can be quantitatively tested with specific tests e.g. the University of Pennsylvania Smell Identification test (UPSIT) or the Brief-Smell Identification test (B-SIT). This evaluation is critical since many patients with cHH and hypo-anosmia are not aware of their defective sense of smell.

Other non-neurological signs are also described, such as midline defects (cleft lip/palate; arched palate), dental abnormalities, renal agenesis/dysgenesis, syndactyly, polydactyly, camptodactyly, and metacarpal shortness. All of these features have to be carefully elicited during the physical examination of these patients and need to be considered all together because some of them can concomitate in GHD patients (**Figure 2**).

Moreover, a deep clinical examination must rule out syndromic conditions associated with hypogonadotropic hypogonadism. For example, the presence of morbid obesity and mental retardation should suggest Prader Willy Syndrome or Bardet-Biedl syndrome. The combination of

coloboma, heart malformations, choanal atresia, growth retardation, genital anomalies should point out a diagnosis of CHARGE syndrome. The presence of 46 XY sex reversal and primary adrenal insufficiency should suggest adrenal hypoplasia congenita secondary to NR0B1 mutation. The presence of other pituitary deficiencies and midline defects should suggest Septo Optic Dysplasia, or the rare Hartsfield syndrome or Gordon Holmes syndrome. (19) (97) The presence of ulnar forearm abnormalities, absence/hypoplasia of mammary and apocrine glands eventually associated with midline defects (i.e. hypoplastic anterior pituitary) or renal malformations in the proband or in a family member may suggest UMS. (31) (**Figure 2**)

3.D ENDOCRINE WORKUP

Basal gonadotropins levels are in the majority of the cases not discriminative. For this reason, other basal endocrine markers had been investigated. Inhibin B, a protein derived from gonadal tissues through FSH stimulation (seminiferous tubules of the testis or cortical layer of the ovaries), are thought to be lower in cHH than in other conditions because of the absence of minipuberty in these patients able to trigger the differentiation either of Sertoli cells in males either of Granulosa cells in females. In males, a low Inhibin B level (≤ 35 pg/mL) (40), or the combination of low Inhibin B levels (10-49 pg/mL) together with low testis volume (< 1 mL) (22), seems to discriminate at best (100% sensitivity and specificity) cHH patients. Moreover, the combination of a low basal LH (< 0.3 IU/L) and a low basal Inhibin B (< 111 pg/ml) has been proven to provide 100% sensitivity and 98% specificity for the cHH diagnosis, giving a valid and reliable alternative to dynamic and more invasive stimulations. (100) In females with cHH, Inhibin B levels are however reported to be either low or normal compared to age-matched controls, therefore not discriminative. (42)

Stimulation test may also be helpful in the diagnostic process. GnRHa Triptorelin proved to have an excellent accuracy for the diagnosis of cHH if LH peaks prove to be lower than 5.3 U/L after 4 hours of pharmacological stimulation (100% sensibility and specificity). (100)

The hCG stimulation test has also been used for many years, primarily to assess the presence of functioning testicular tissue and to investigate defects of testosterone biosynthesis and action. It is based on the ability of hCG to increase androgen production in Leydig cells via stimulation of the LH receptor. Therefore, a blunted testosterone response to hCG stimulation test has been supposed as evidence towards a cHH diagnosis. However, many protocols have been used and large studies are lacking. Promising results have been assumed with longer protocols (19 days of stimulation proving to perform better than 3 days) (45) or higher doses (subsequent injections of 1500 UI of hCG in three consecutive days) (46). Combining the results of GnRH test stimulation and hCG test has also proved to differentiate cHH from CDGP with a sensibility and a specificity of 100% if LH peaks under LHRH test are < 2.8 U/L, testosterone levels after 3 day hCG test are < 3.6 nmol/L and testosterone levels after 19 days of hCG test are < 9.5 nmol/L. (45)

3.E GENETIC TESTING

The pathogenesis of cHH, with or without hypo/anosmia, is a wide area of current research in endocrinology. A genetic basis of cHH can be defined in almost 50% of the cases. (97) The genetic heterogeneity of cHH is due to the fact that all modes of inheritance can occur: X-linked, autosomal recessive and autosomal dominant. Rising complexity to the picture, the traditional Mendelian view of inheritance has been revised following identification of oligogenic forms of cHH in at least 20% of the cases. (2) Moreover, a specific genotype-phenotype correlation is frequently lacking, as the penetrance is incomplete and the expressivity extremely variable, again possibly due to the oligogenic fashion of inheritance, although an involvement of environmental factors cannot be excluded. Many mutations in genes known to cause aberrations of the hypothalamic-pituitary-gonadal axis have been described. The mutations can be classified as either affecting GnRH action, GnRH secretion, GnRH neurons development and migration or GnRH neurons specification and/or activation, and many of them are shared with CDGP cohorts. Moreover, some mutation in genes responsible for hereditary

forms of MPHD or syndromic conditions have also been associated with both cHH and GHD. (97)
The genetic basis of cHH and shared causes with CDGP or GHD cohort are illustrated in **Figure 1**.

3.F FINAL OUTCOMES

For males, treatment is based on the use of testosterone esters given either by intramuscular or transdermal routes with subsequent gradual dose increase every 6 months until reaching, in around 36 months, the adult scheme. The use of testosterone, either injectable or transdermal, leads to good virilization but has little-no effect on testicular growth. Treatment for females with hypogonadism is based on the initial use of estrogens in the form of 17 β -estradiol (preferably), either trans dermally (fancied) or orally, or in the form of oral EE. Estrogen therapy must also be integrated with progestin therapy after at least 2 years (or earlier in the case of more than one significant bleeding episode) in order to avoid endometrial hypertrophy. The natural (i.e. micronized) over the synthetic forms of progestins are preferred. A complete explanation of therapeutic protocols and doses is beyond the scope of this review but can be found in the recent review published by Raivio T. et al. (3) While testosterone replacement only causes virilization, treatment with recombinant FSH associated with hCG has been shown to induce testes growth, increase Inhibin B levels and stimulate spermatogenesis in adolescent males with cHH. Moreover, an early treatment with gonadotropins has been suggested to have a positive effect on future fertility. (44) Also, as already mentioned, the observation of a self limiting pubertal progression at discontinuation of sex steroids therapy may point out a cHH rather than CDGP diagnosis. (3)

EVIDENCE SYNTHESIS

In every child referred for a suspected growth disorder, anthropometric measurements, medical history (with special attention to the so called “red flag”, see **Figure 2**), full physical examination

with special attention to dysmorphisms and disproportions, pubertal development and bone age assessment must be evaluated by the pediatric endocrinologist.

Regarding auxological measurements standing height, arm span, sitting height and leg length must be measured at every examination, with the use of a proper stadiometer and a tape-measure. At the same time pubertal stage must be evaluated by the use of Tanner charts, with the use of a proper orchidometer for boys. At this regard it is worth remembering that, even though in both sexes hair growth in the pubic area depends on adrenal secretion, clinically, a delayed gonadarche together with a delayed adrenarche and pubarche orients towards a CDGP diagnosis, whereas delayed gonadal development alone is a more peculiar presentation of cHH patients. (25)

Bone age is another important diagnostic tool. To this extent it is worth remembering that in every child, TW3 method and G&P method may overestimate and underestimate bone age, respectively. (28) Moreover, because G&P standards and TW standards were derived from white children living in the United States and predominantly of North European ancestry, the use of new automated methods is recommended for the assessment of children of other ethnicity groups. (27) Bone age is usually found to be delayed in relation to chronological age in all three CDGP, GHD and cHH patients. Even if many endocrine (hypothyroidism, rickets, Cushing disease, panhypopituitarism) and non-endocrine conditions (malnutrition, prematurity, cardiac, liver or kidney diseases, systemic inflammations or infections) may present with a delayed bone age, this assessment is considered a non-invasive and cost-effective method able to select those patients in which further testing are recommended. In fact, a great proportion of patients with non-endocrine short stature (familial short stature, skeletal dysplasia, idiopathic short stature) may be excluded from further testing because displaying a bone age equal or sometimes advanced compared to chronological age, or because manifesting straightforward signs of skeletal dysplasia on X-rays. Moreover, usually, a failure of spontaneous pubertal development at the bone age maturation of 12 and 13 years, in females and males respectively, may be indicative of an organic cause rather than CDGP. (28)

Bone age is also a useful starting point to predict FH outcomes. (**Table II**) PAH may be helpful for the differential diagnosis, as it will end up laying in the lower end of target height for CDGP patients, be far way lower than TH in GHD children, hence higher than TH in cHH patients. (**Table I**).

Taken as a whole, self-limited delayed puberty is the most common cause of short stature and delayed puberty in both sexes in clinical practice, and the higher number of males that present to medical attention is thought to be a consequence of referral bias. According to the literature, we can expect to find an underlying secondary medical cause of poor growth/delayed puberty in up to 27% of boys and 57% of girls presenting to medical attention. (3) Chronic systemic illnesses and malnutrition (the so called “functional causes” of delayed puberty) and other causes of poor growth must be ruled out by baseline blood exams including full blood count, liver function test, creatinine, celiac screen, thyroid function tests, IGF-1, PRL, LH/FSH, testosterone/estradiol levels. (25) At the same time, the evidence of syndromic stigmata may evoke a specific syndromic phenotype or a contiguous gene syndrome. The targeted genome analysis and/or a standard karyotype together with an array-CGH may be useful investigations in these situations. When all these confounding phenotypes are ruled out, the diagnosis must be carried out through a step-by-step approach (**Figure 3**).

Useful information can firstly be taken from growth charts from infancy, at least in boys. CDGP boys soon after birth are known to display poor growth until 2 years old, then tend to level till 5 years old, starting from when a height SDS loss starts to be detectable reaching their nadir at puberty (26) and BMI is found to be concordantly low. (30) By contrast, in boys with GHD a continuous decrease in height SDS without any levelling is found from birth on and a severe short stature is frequently detected (68), together with an increase in fat mass (in patients left untreated). (30) In boys with cHH, instead, height SDS does not differ to that of target potential in the first 5 years of life but a typical decreases at puberty occurs, with an inverse correlation with BMI. (30) Special considerations must then be taken for girls, in which GV does not discriminate at best the different etiologies of delayed puberty. (22)

If cHH presents with pathognomonic “red flags” (e.g. anosmia, eunuchoid habitus, pituitary lesions, ulnar-mammary malformations) this should help in the diagnostic process, however many features seem to overlap with GHD cohorts, further mistaking the diagnosis (**Figure 2**) .

Anyway, not all peripubertal children with pathological causes of growth failure present to medical attention with short stature. If insufficient clues for a disturbed growth are found at the first screening, the patient can be put on watchful waiting. If clues for disturbed growth are highlighted, additional further investigations must be performed. A combination of short stature ($\text{SDS H} < -2.5$), severe distance to target height ($\Delta \text{SDS H-TH} > -1.6$) and growth deflection ($>0.5 \text{ SDS/year}$ or $> 0.7 \text{ SDS/2 years}$ or $> 1 \text{ SDS/undefined time}$) should be used as practical guideline to decide which child should be screened with a GHST. (17)

An important consideration must be taken for those children in which puberty may be anticipated: in this scenario growth velocity may be falsely reassuring because driven by pubertal hormones, but then a severe deviation from target height becomes evident after puberty completion if growth hormone deficiency concomits. For this reason patients at risk of having GHD (i.e. Cancer Survivors treated with surgery and/or total body radiation and/or chemotherapy) must be investigated with a growth hormone stimulation test regardless of height SDS and GV SDS. (33)

In terms of GHST, hypothalamic stimulation (hypoglycemia tolerance test, clonidine test, arginine test, glucagon test) are preferred rather than combined stimuli (i.e. GHRH+ arginine) in order to rule out possible hypothalamic causes of growth hormone deficiency. Moreover, priming with sex steroids before GHST is useful in order to increase diagnostic accuracy of the stimulation and reduce the false positive diagnosis of presumed peripubertal idiopathic GHD, at least for boys > 11 years old and for girls > 10 years old. (32) To this extent many protocols exist within different centers (39): the use of 100 mg of testosterone enanthate intramuscularly 1 week before the test for boys, and 1-2 mg ($< 20 \text{ kg}$ or $> 20 \text{ kg}$ respectively) of valerate estradiol orally for girls for the three nights before the test may

be considered a reasonable scheme, even though oral estradiol preparations can also be used for boys if a less invasive route of administration is preferred. If GH peak under GHST is less than 7-8 $\mu\text{g/L}$ (with immunometric assays), especially under 2 different stimulations, the child is considered as having GHD and a pituitary MRI is warranted. If no tumoral lesions in the brain are found, rhGH therapy can be started at substitutive doses (25-35 $\mu\text{g/kg/die}$) and the child must be followed up periodically in order to verify appropriate catch-up growth after rhGH is commenced. According to the severity of GHD and to the underlying cause, early retesting of GH secretion may be considered. (95) (96)

It is also worth mentioning that in terms of final height outcomes a lot of different results are described in literature for treated GHD patients, showing a FH SDS spanning between -1.1 to -0.5 and ΔSDS FH -TH concordantly ranging between -0.6 to +1.08. For this reason, it is also very difficult to confirm the diagnosis backwards, as these results overlap mostly with those described in some CDGP cohorts (**Tables III and IV**). This is possibly due to the low specificity of GHST per se together with the fact that most of these studies were performed without any priming preparation before stimulation, and as a result they might include also some false positive patients that could have possibly displayed normal GH peaks if sex steroids priming would had been performed.

If GH peaks prove instead to be higher than 7-8 $\mu\text{g/L}$ in at least one GHST, a diagnosis of CDGP or cHH may be expected. The differential diagnosis between these two conditions is however far from being straightforward, especially in the peripubertal age given the heterogeneity of these diseases and the tendency to overlap of one into another clinically, hormonally and genetically. (**Figures 1 and 2**).

Basal gonadotropin levels have limited diagnostic specificity as they tend to be low both in CDGP and cHH cohorts. Even if in some CDGP patients at the very beginning of puberty gonadotropins may be detectable ($\text{LH} > 0.2\text{-}0.3 \text{ U/L}$), this picture again tends to overlap at least with partial forms of cHH. (43)

For this reason, dynamic stimulation of HPG axis has been proposed. A combination of LHRH test and hCG test (3days & 19 days stimulation) has been proposed in order to increase sensitivity and specificity of cHH diagnosis by 100%. (45)

At least in boys, Sertoli cell markers but not Leydig cells, growth or adrenal markers are considered helpful in establishing whether a boy with delayed puberty will progress spontaneously. (41) Inhibin B may provide a simple first-line test capable of identifying a subset of patients with delayed puberty who are highly likely to have cHH. These test may be performed before a short period of treatment with LDSS, which is considered more indicative and helpful. In fact, an increase in growth velocity and in gonadal and breast development after a short course of 3-6 months of LDSS can be taken as evidence against the presence of GHD or cHH. (3) (64) This treatment may at the same time be helpful for CDGP children in sensibilizing their anterior pituitary glands to sex steroids in order to allow puberty to trigger spontaneously, minimizing psychosocial distress of these patients compared to peers and avoiding the known long term metabolic and bone sequelae associated with delayed puberty. (2)

Finally, it is worth mentioning that as these conditions may overlap mostly in terms of genetic causes, this is not yet a field able to differentiate these conditions, even though some promising studies on specific genes selectively discovered in CDGP families have been recently published. (19)

CONCLUSIONS

After systemic chronic illnesses or other causes of “functional” delayed growth have been ruled out, CDGP may be the most probable cause of poor growth in a child presenting with short stature and delayed puberty, especially for boys. However the differential diagnosis between CDGP, GHD and cHH is a challenging one for the clinician at the beginning of clinical examination, for many reasons. First, as already mentioned, children may present with the same auxological features (short stature, low growth velocity, delayed puberty, delayed bone age). Secondary, even if PAH may help, it is difficult to reliably trust the predicted adult height given the low accuracy of different methods, especially if bone age is extremely delayed. Third, although the presence of a positive familial history of delayed puberty may give a cue towards a presumptive CDGP diagnosis, cHH cannot be excluded giving the high heterogeneity of cHH presentation within pedigrees. Fourth, because of the lack of sensibilization of the pituitary gland of these children to detectable levels of sex steroids, peripubertal CDGP can display decreased GH stimulation peaks under GHST and lower IGF-1 levels compared with their pubertal, age-matched peers, pointing out the need of further investigations in order to rule out a low pituitary growth hormone reserve. For this reason we recommend the use of priming before GHST in every patient tested for GHD at least from the age of 10 years old (girls) or 11 years old (boys). A treatment hormonal approach is also recommended afterwards for those patients with normal GH peaks under GHST in order to allow a correct differential diagnosis between CDGP and cHH, being beneficial at the same time for psychosocial wellness, final height outcomes, sexual, bone and metabolic health.

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REFERENCES

1. Barinaga M, Yamamoto G, Rivier C, Vale W, Evans R, Rosenfeld MG. Transcriptional regulation of growth hormone gene expression by growth hormone-releasing factor. *Nature* 1983; 306(5938): 84–5.
2. Boehm U, Bouloux P-M, Dattani MT, de Roux N, Dodé C, Dunkel L, et al. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism--pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* 2015;11(9):547–64.
3. Raivio T, Miettinen PJ. Constitutional delay of puberty versus congenital hypogonadotropic hypogonadism: Genetics, management and updates. *Best Pract Res Clin Endocrinol Metab* 2019;33(3):1013-16.
4. Weissberger AJ, Ho KK. Activation of the somatotrophic axis by testosterone in adult males: evidence for the role of aromatization. *J Clin Endocrinol Metab* 1993;76(6):1407–12.
5. Veldhuis JD, Metzger DL, Martha PM, Mamas N, Kerrigan JR, Keenan B, et al. Estrogen and testosterone, but not a nonaromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. *J Clin Endocrinol Metab* 1997;3414–20.
6. Meinhardt UJ, Ho KKY. Modulation of growth hormone action by sex steroids. *Clin Endocrinol (Oxf)* 2006; 65(4):413–22.
7. Hartmann BW, Huber JC, Kirchengast S, Söregi, M.D. G, Albrecht AE. Effect of

hormone replacement therapy on growth hormone stimulation in women with premature ovarian failure. *Fertil Steril* 1997; 68(1):103–7.

8. González-Parra S, Argente J, García-Segura LM, Chowen JA. Cellular Composition of the Adult Rat Anterior Pituitary Is Influenced by the Neonatal Sex Steroid Environment. *Neuroendocrinology* 1998;68(3):152–62.
9. Yan M, Jones MEE, Hernandez M, Liu D, Simpson ER, Chen C. Oestrogen Replacement in vivo Rescues the Dysfunction of Pituitary Somatotropes in Ovariectomised Aromatase Knockout Mice. *Neuroendocrinology* 2005;81(3):158–66.
10. Mauras N, Rogol AD, Haymond MW, Veldhuis JD. Sex steroids, growth hormone, insulin-like growth factor-1: neuroendocrine and metabolic regulation in puberty. *Horm Res* 1996;45(1–2):74–80.
11. Albertsson-Wikland K, Rosberg S, Libre E, Lundberg LO, Groth T. Growth hormone secretory rates in children as estimated by deconvolution analysis of 24-h plasma concentration profiles. *Am J Physiol Metab.* 1989;257(6):E809–14.
12. Giustina A, Scalvini T, Tassi C, Desenzani P, Poiesi C, Wehrenberg WB, et al. Maturation of the regulation of growth hormone secretion in young males with hypogonadotropic hypogonadism pharmacologically exposed to progressive increments in serum testosterone. *J Clin Endocrinol Metab.* 1997;82(4):1210–9.
13. Mansfield MJ, Rudlin cCR, Crigler JF, Karol KA, Crawford JD, Boepple PA, et al. Changes in Growth and Serum Growth Hormone and Plasma Soipatomedin-C Levels During Suppression of Gonadal Sex Steroid Secretion in Girls with Central Precocious Puberty. *J Clin Endocrinol Metab* 1988;66(1):3–9.
14. Marin G, Domené HM, Barnes KM, Blackwell BJ, Cassorla FG, Cutler GB. 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 Clin Endocrinol Metab* 1994;79(2):537–41.

15. Wit JM, Ranke MB KC. International Classification of Paediatric Endocrine Diagnoses. *Hormon Res. Pediatr.* 2016; 86(3): 212-214.
16. Lawaetz JG, Hagen CP, Mieritz MG, Jensen MB, Petersen JH, Juul A. Evaluation of 451 Danish boys with delayed puberty: Diagnostic use of a new puberty nomogram and effects of oral testosterone therapy. *J Clin Endocrinol Metab.* 2015;100(4):1376–85.
17. Stalman SE, Hellinga I, Wit JM, Hennekam RCM, Kamp GA, Plötz FB. Growth failure in adolescents: etiology, the role of pubertal timing and most useful criteria for diagnostic workup. *J Pediatr Endocrinol Metab* 2016;29(4):465–73.
18. Cassatella D, Howard SR, Acierno JS, Xu C, Papadakis GE, Santoni FA, et al. Congenital hypogonadotropic hypogonadism and constitutional delay of growth and puberty have distinct genetic architectures. *Eur J Endocrinol* 2018;178(4):377–88.
19. Howard SR. The genetic basis of delayed puberty, *Frontiers in Endocrinology*. Frontiers Media S.A. 2019; (10): 423.
20. Reindollar RH, Rogers Byrd J, McDonough PG. Delayed sexual development: A study of 252 patients. *Am J Obstet Gynecol* 1981;140(4):371–80.
21. Grimberg A, Feemster KA, Pati S, Ramos M, Grundmeier R, Cucchiara AJ, et al. Medically underserved girls receive less evaluation for short stature. *Pediatrics.* 2011;127(4):696–702.

- 1 22. Varimo T, Miettinen PJ, Käsäkoski J, Raivio T HM. Congenital hypogonadotropic
2 hypogonadism, functional hypogonadotropism or constitutional delay of growth and
3 puberty? An analysis of a large patient series from a single tertiary center. Hum
4 Reprod 2017;32(1):147–53.
5
6
7
8
9
- 10 23. Wudy SA, Hagemann S, Dempfle A, Ringler G, Blum WF, Berthold LD, et al.
11 Children with idiopathic short stature are poor eaters and have decreased body
12 mass index. Pediatrics 2005; 116(1):52-7.
13
14
15
16
17
- 18 24. Han JC, Balagopal P, Sweeten S, Darmaun D, Mauras N. Evidence for
19 hypermetabolism in boys with constitutional delay of growth and maturation. J Clin
20 Endocrinol Metab. 2006;91(6):2081–6.
21
22
23
24
- 25 25. Howard SR, Dunkel L. Delayed Puberty—Phenotypic Diversity, Molecular Genetic
26 Mechanisms, and Recent Discoveries. Endocr Rev. 2019;40(5):1285–317.
27
28
29
30
- 31 26. Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an
32 academic center. J Clin Endocrinol Metab 2002;87(4):1613–20.
33
34
35
36
- 37 27. Ren X, Li T, Yang X, Wang S, Ahmad S, Xiang L, et al. Regression Convolutional
38 Neural Network for Automated Pediatric Bone Age Assessment from Hand
39 Radiograph. IEEE J Biomed Heal Informatics 2019;23(5):2030–8.
40
41
42
43
- 44 28. Creo AL, Schwenk WF. Bone age: A handy tool for pediatric providers Pediatrics.
45 American Academy of Pediatrics 2017; 140(6):e20171486
46
47
48
49
- 50 29. Brämswig JH, Fasse M, Holthoff M-L, von Lengerke HJ, von Petrykowski W,
51 Schellong G. Adult height in boys and girls with untreated short stature and
52 constitutional delay of growth and puberty: Accuracy of five different methods of
53 height prediction. J Pediatr 1990;117(6):886–91.
54
55

- 1 30. Reinehr T, Hoffmann E, Rothermel J, Lehrian TJ, Brämwig J, Binder G. A New
2 Model of Adult Height Prediction Validated in Boys with Constitutional Delay of
3 Growth and Puberty. *Horm Res Paediatr* 2019;91(3):186–94.
4
5
6
7
8
9 31. Galazzi E, Duminuco P, Moro M, Guizzardi F, Marazzi N, Sartorio A, et al.
10 Hypogonadotropic hypogonadism and pituitary hypoplasia as recurrent features in
11 ulnar-mammary syndrome. *Endocr Connect*. 2018;7(12) 1432-1441.
12
13
14
15
16 32. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, et al.
17 Guidelines for Growth Hormone and Insulin-Like Growth Factor-I Treatment in
18 Children and Adolescents: Growth Hormone Deficiency, Idiopathic Short Stature,
19 and Primary Insulin-Like Growth Factor-I Deficiency. *Horm Res Paediatr*. 2017;86(6)
20 361–97.
21
22
23
24
25
26
27 33. Collett-Solberg PF, Ambler G, Backeljauw PF, Bidlingmaier M, Biller BMK,
28 Boguszewski MCS, et al. Diagnosis, Genetics, and Therapy of Short Stature in
29 Children: A Growth Hormone Research Society International Perspective. *Horm Res*
30 *Paediatr*. 2019;92(1):1–14.
31
32
33
34
35
36 34. Martínez AS, Domené HM, Ropelato MG, Jasper HG, Pennisi PA, Escobar ME, et
37 al. Estrogen Priming Effect on Growth Hormone (GH) Provocative Test: A Useful
38 Tool for the Diagnosis of GH Deficiency. *J Clin Endocrinol Metab*.
39 2000;85(11):4168–72.
40
41
42
43
44
45 35. Molina S, Paoli M, Camacho N, Arata-Bellabarba G, Lanes R. Is Testosterone and
46 Estrogen Priming Prior to Clonidine Useful in the Evaluation of the Growth Hormone
47 Status of Short Peripubertal Children? *J Pediatr Endocrinol Metab*. 2008;21(3) 257-
48 66.
49
50
51
52
53
54 36. Müller G, Keller A, Reich A, Hoepffner W, Kratzsch J, Buckler JM, et al. Priming with
55

Testosterone Enhances Stimulated Growth Hormone Secretion in Boys with Delayed Puberty. *J Pediatr Endocrinol Metab* 2004;17(1) 77-83.

37. Soliman A, Adel A, Sabt A, Elbukhari E, Ahmed H, Sanctis V. Does priming with sex steroids improve the diagnosis of normal growth hormone secretion in short children? *Indian J Endocrinol Metab* 2014;18(7):80-3.
38. Gonc EN, Kandemir N, Ozon A, Alikasifoglu A. Final heights of boys with normal growth hormone responses to provocative tests following priming. *J Pediatr Endocrinol Metab* 2008;21(10):963-71.
39. Murray PG, Dattani MT, Clayton PE. Controversies in the diagnosis and management of growth hormone deficiency in childhood and adolescence *Archives of Disease in Childhood*. 2016;101:96-100.
40. Coutant R, Biette-Demeneix E, Bouvattier C, Bouhours-Nouet N, Gatelais F, Dufresne S, et al. Baseline inhibin B and anti-Mullerian hormone measurements for diagnosis of hypogonadotropic hypogonadism (HH) in boys with delayed puberty. *J Clin Endocrinol Metab* 2010;95(12):5225-32.
41. Rohayem J, Nieschlag E, Kliesch S, Zitzmann M. Inhibin B, AMH, but not INSL3, IGF1 or DHEAS support differentiation between constitutional delay of growth and puberty and hypogonadotropic hypogonadism. *Andrology* 2015;3(5):882-7.
42. Li HWR, Anderson RA, Yeung WSB, Ho PC, Ng EHY. Evaluation of serum antimullerian hormone and inhibin B concentrations in the differential diagnosis of secondary oligoamenorrhea. *Fertil Steril* 2011;96(3):774-9.
43. Resende EAMR, Lara BHJ, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of Basal and Gonadotropin-Releasing Hormone-Stimulated

Gonadotropins by Immunochemiluminometric and Immunofluorometric Assays in Normal Children. *J Clin Endocrinol Metab* 2007;92(4):1424–9.

44. Harrington J, Palmert MR. Clinical review: Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. *J Clin Endocrinol Metab* 2012;97(9):3056–67.
45. Segal TY, Mehta A, Anazodo A, Hindmarsh PC, Dattani MT. Role of Gonadotropin-Releasing hormone and human chorionic gonadotropin stimulation tests in differentiating patients with hypogonadotropic hypogonadism from those with constitutional delay of growth and puberty. *J Clin Endocrinol Metab* 2009;94(3):780–5.
46. Kauschansky A, Dickerman Z, Phillip M, Weintrob N, Strich D. Use of GnRH agonist and human chorionic gonadotrophin tests for differentiating constitutional delayed puberty from gonadotrophin deficiency in boys. *Clin Endocrinol (Oxf)* 2002; 56(5) 603–7.
47. Chan YM, Lippincott MF, Kusa TO, Seminara SB. Divergent responses to kisspeptin in children with delayed puberty. *JCI insight*. 2018;3(8) e99109.
48. Dunkel L, Quinton R. Transition in endocrinology: Induction of puberty. Vol. 170, *European Journal of Endocrinology*. BioScientifica Ltd. 2014; R229-39.
49. Sukumar SP, Bhansali A, Sachdeva N, Ahuja CK, Gorski U, Jarial KDS, et al. Diagnostic utility of testosterone priming prior to dynamic tests to differentiate constitutional delay in puberty from isolated hypogonadotropic hypogonadism. *Clin Endocrinol (Oxf)* 2017;86(5):717–24.
50. Taha D, Mullis PE, Ibáñez L, De Zegher F. Absent or delayed adrenarche in Pit1/

POU1F1 deficiency. *Horm Res* 2005; 64(4):175–9.

51. Pugliese-Pires PN, Fortin JP, Arthur T, Latronico AC, Mendonca BB, Villares SMF, et al. Novel inactivating mutations in the GH secretagogue receptor gene in patients with constitutional delay of growth and puberty. *Eur J Endocrinol* 2011;165(2):233–41.
52. Day FR, Elks CE, Murray A, Ong KK, Perry JRB. Puberty timing associated with diabetes, cardiovascular disease and also diverse health outcomes in men and women: The UK Biobank study. *Sci Rep*. 2015;(5):11208.
53. Bourguignon JP. Variations in duration of pubertal growth: a mechanism compensating for differences in timing of puberty and minimizing their effects on final height. Belgian Study Group for Paediatric Endocrinology. *Acta Paediatr Scand Suppl* 1988;347:16–24.
54. Crowne EC, Shalet SM, Wallace WH, Eminson DM, Price DA. Final height in boys with untreated constitutional delay in growth and puberty. *Arch Dis Child* [Internet]. 1990;65(10):1109–12.
55. Poyrazoğlu Ş, Günöz H, Darendeliler F, Saka N, Bundak R, Baş F. Constitutional delay of growth and puberty: From presentation to final height. *J Pediatr Endocrinol Metab*. 2005;18(2):171–9.
56. Couto-Silva AC, Trivin C, Adan L, Lawson-Body E, Souberbielle JC, Brauner R. Management of boys with short stature and delayed puberty. *J Pediatr Endocrinol Metab* 2005;18(8):807–13.
57. Zucchini S, Wasniewska M, Cisternino M, Salerno M, Iughetti L, Maghnie M, et al. Adult height in children with short stature and idiopathic delayed puberty after

different management. *Eur J Pediatr* 2008 Jun 24;167(6):677–81.

58. Martin MM, Martin ALA, Mossman KL. Testosterone treatment of constitutional delay in growth and development: effect of dose on predicted versus definitive height. *Acta Endocrinol (Copenh)* 1986;113(4_Suppl):S147–52.
59. Uruena M, Pantiotou S, Preece MA, Stanhope R. Is testosterone therapy for boys with constitutional delay of growth and puberty associated with impaired final height and suppression of the hypothalamo-pituitary-gonadal axis? *Eur J Pediatr* 1992;151(1):15–8.
60. Kohva E, Varimo T, Huopio H, Tenhola S, Voutilainen R, Toppari J, et al. Anti-Müllerian hormone and letrozole levels in boys with constitutional delay of growth and puberty treated with letrozole or testosterone. *Hum Reprod* 2020;35(2):257–64.
61. Stanhope R, Buchanan CR, Fenn GC, Preece MA. Double blind placebo controlled trial of low dose oxandrolone in the treatment of boys with constitutional delay of growth and puberty. *Arch Dis Child* 1988;63(5):501–5.
62. De Luca F, Argente J, Cavallo L, Crowne E, Delemarre-Van de Waal HA, De Sanctis C, et al. Management of Puberty in Constitutional Delay of Growth and Puberty. *J Pediatr Endocrinol Metab* 2001;14(s2):953–7.
63. Crowne EC, Shalet SM, Wallace WH, Eminson DM, Price DA. Final height in girls with untreated constitutional delay in growth and puberty. *Eur J Pediatr* 1991;150(10):708–12.
64. Kaplowitz PB. Diagnostic value of testosterone therapy in boys with delayed puberty. *Am J Dis Child* 1989;143(1):116–20.

- 1 65. Chioma L, Papucci G, Fintini D, Cappa M. Use of testosterone gel compared to
2 intramuscular formulation for puberty induction in males with constitutional delay of
3 growth and puberty: a preliminary study. *J Endocrinol Invest*. 2018 Feb 1;41(2):259–
4 63.
5
6
7
8
9
10 66. Overview | Human growth hormone (somatropin) for the treatment of growth failure
11 in children | Guidance | NICE. [Internet]. Available from
12 <https://www.nice.org.uk/guidance/TA188> [cited 2020 January 29]
13
14
15
16
17 67. Murray PG, Clayton PE. Disorders of Growth Hormone in Childhood. In: Feingold
18 KR, Anawalt B, Boyce A, et al., (Editors) *Endotext*. South Dartmouth (MA):
19 MDTText.com, Inc.; 2000. Available from:
20 <https://www.ncbi.nlm.nih.gov/books/NBK278971>
21
22
23
24
25
26 68. Rothermel J, Lass N, Toschke C, Reinehr T. Progressive decline in height standard
27 deviation scores in the first 5 years of life distinguished idiopathic growth hormone
28 deficiency from familial short stature and constitutional delay of growth. *Horm Res*
29 *Paediatr*. 2016;86(2):117–25.
30
31
32
33
34
35 69. Biczysko-Mokosa A, Petriczko E, Horodnicka-Józwa A, Dawid G, Kedzia A, Lewiński
36 A, et al. Evaluation of final height prediction and selected parameters in Polish
37 patients with severe and partial growth hormone deficiency. *Neuro Endocrinol Lett*.
38 2014;35(3):242–7.
39
40
41
42
43
44 70. Inoue-Lima TH, Vasques GA, Scalco RC, Nakaguma M, Mendonca BB, Arnhold IJP,
45 et al. IGF-1 assessed by pubertal status has the best positive predictive power for
46 GH deficiency diagnosis in peripubertal children. *J Pediatr Endocrinol Metab*. 2019
47 Feb 1;32(2):173–9.
48
49
50
51
52
53 71. Blum WF, Alherbish A, Alsagheir A, El Awwa A, Kaplan W, Koledova E, et al. The
54
55

growth hormone-insulin-like growth factor-I axis in the diagnosis and treatment of growth disorders. *Endocrine Connections*. BioScientifica Ltd. 2018;(7):R212–22.

72. Ghigo E, Bellone J, Aimaretti G, Bellone S, Loche S, Cappa M, et al. Reliability of provocative tests to assess growth hormone secretory status. Study in 472 normally growing children. *J Clin Endocrinol Metab* 1996;81(9):3323–7.
73. Giordano M. Genetic causes of isolated and combined pituitary hormone deficiency. *Best Pract Res Clin Endocrinol Metab* 2016;30(6):679–91.
74. Léger J, Danner S, Simon D, Garel C, Czernichow P. Do all patients with childhood-onset growth hormone deficiency (GHD) and ectopic neurohypophysis have persistent GHD in adulthood? *J Clin Endocrinol Metab* 2005;90(2):650–6.
75. Quigley CA, Zagar AJ, Liu CC, Brown DM, Huseman C, Levitsky L, et al. United States multicenter study of factors predicting the persistence of GH deficiency during the transition period between childhood and adulthood. *Int J Pediatr Endocrinol* 2013;(1):6.
76. Tauber M, Moulin P, Pienkowski C, Jouret B, Rochiccioli P. Growth hormone (GH) retesting and auxological data in 131 GH-deficient patients after completion of treatment. *J Clin Endocrinol Metab* 1997;82(2):352–6.
77. Toogood A, Shalet S. Diagnosis of severe growth hormone (GH) deficiency in young adults who received GH replacement therapy during childhood. *Acta Paediatr* 1997;86(S423):117–20.
78. Donaubauer J, Kiess W, Kratzsch J, Nowak T, Steinkamp H, Willgerodt H, et al. Re-assessment of growth hormone secretion in young adult patients with childhood-onset growth hormone deficiency. *Clin Endocrinol (Oxf)* 2003;58(4):456–63.

- 1 79. Murray PG, Hague C, Fafoula O, Gleeson H, Patel L, Banerjee I, et al. Likelihood of
2 persistent GH deficiency into late adolescence: relationship to the presence of an
3 ectopic or normally sited posterior pituitary gland. Clin Endocrinol (Oxf)
4 2009;71(2):215–9.
5
6
7
8
9
10 80. Yadav P, Singhal S, Chauhan S, Harit S. MRI evaluation of size and shape of
11 normal pituitary gland: age and sex related changes." J Clin Diagn Res 2017 TC01-
12 TC04.
13
14
15
16
17 81. Argyropoulou M, Perignon F, Brunelle F, Brauner R, Rappaport R. Height of normal
18 pituitary gland as a function of age evaluated by magnetic resonance imaging in
19 children. Pediatr Radiol 1991;21(4):247–9.
20
21
22
23
24 82. Han X, Xiu J, Huang Z, Zhang J, Zhang Z, Dong Y, et al. Three-dimensional
25 magnetic resonance volumetry of the pituitary gland is effective in detecting short
26 stature in children. Exp Ther Med 2014;8(2):551–6.
27
28
29
30
31
32 83. Doraiswamy PM, Potts JM, Axelson DA, Husain MM, Lurie SN, Na C, et al. MR
33 assessment of pituitary gland morphology in healthy volunteers: age- and gender-
34 related differences. AJNR Am J Neuroradiol;13(5):1295–9.
35
36
37
38
39 84. Kessler M, Tenner M, Frey M, Noto R. Pituitary volume in children with growth
40 hormone deficiency, idiopathic short stature and controls. J Pediatr Endocrinol
41 Metab 2016;29(10):1195–200.
42
43
44
45
46
47 85. Maghnie M, Lindberg A, Koltowska-Häggström M, Ranke MB. Magnetic resonance
48 imaging of CNS in 15043 children with GH deficiency in KIGS (Pfizer International
49 Growth Database). Eur J Endocrinol 2013;168(2):211–7.
50
51
52
53
54 86. Krström B, Aronson AS, Dahlgren J, Gustafsson J, Halldin M, Ivarsson SA, et al.
55

Growth Hormone (GH) Dosing during Catch-Up Growth Guided by Individual Responsiveness Decreases Growth Response Variability in Prepubertal Children with GH Deficiency or Idiopathic Short Stature. *J Clin Endocrinol Metab* 2009;94(2):483–90.

87. Reiter EO, Price DA, Wilton P, Albertsson-Wikland K, Ranke MB. Effect of growth hormone (GH) treatment on the near-final height of 1258 patients with idiopathic GH deficiency: analysis of a large international database. *J Clin Endocrinol Metab* 2006;91(6):2047–54.
88. Cutfield W, Lindberg A, Wikland KA, Chatelain P, Ranke M, Wilton P. Final height in idiopathic growth hormone deficiency: the KIGS experience. *Acta Paediatr* 1999;88(s428):72–5.
89. Blethen SL, Baptista J, Kuntze J, Foley T, LaFranchi S, Johanson A. Adult Height in Growth Hormone (GH)-Deficient Children Treated with Biosynthetic GH. *J Clin Endocrinol Metab* 1997;82(2):418–20.
90. Pozzobon G, Partenope C, Mora S, Garbetta G, Weber G, Barera G. Growth hormone therapy in children: predictive factors and short-term and long-term response criteria. *Endocrine* 2019; 66(3):614–621
91. Deodati A, Cianfarani S. Impact of growth hormone therapy on adult height of children with idiopathic short stature: systematic review. *BMJ* 2011;(342):c7157.
92. Wit JM, Ranke MB, Albertsson-Wikland K, Carrascosa A, Rosenfeld RG, Van Buuren S, et al. Personalized approach to growth hormone treatment: Clinical use of growth prediction models. Vol. 79, *Hormone Research in Paediatrics. Horm Res Paediatr*; 2013; 257–70.

- 1 93. Yuen KCJ, Biller BMK, Radovick S, Carmichael JD, Jasim S, Pantalone KM, et al.
2 American association of clinical endocrinologists and american college of
3 endocrinology guidelines for management of growth hormone deficiency in adults
4 and patients transitioning from pediatric to adult care. *Endocr Pract*
5 2019;25(11):1191–232.
6
7
8
9
10
11
12 94. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, et al.
13 Guidelines for Growth Hormone and Insulin-Like Growth Factor I Treatment in
14 Children and Adolescents: Growth Hormone Deficiency, Idiopathic Short Stature,
15 and Primary Insulin-Like Growth Factor-I Deficiency. *Horm Res Paediatr*
16 2016;86(6):361–97.
17
18
19
20
21
22
23 95. Loche S, Bizzarri C, Maghnie M, Faedda A, Tziella C, Autelli M, et al. Results of
24 early reevaluation of growth hormone secretion in short children with apparent
25 growth hormone deficiency. *J Pediatr* 2002;140(4):445–9.
26
27
28
29
30 96. Vuralli D, Gonc EN, Ozon ZA, Alikasifoglu A, Kandemir N. Clinical and laboratory
31 parameters predicting a requirement for the reevaluation of growth hormone status
32 during growth hormone treatment: Retesting early in the course of GH treatment.
33 *Growth Horm IGF Res* 2017;34:31–7.
34
35
36
37
38
39
40
41 97. Cangiano B, Swee DS, Quinton R, Bonomi M. Genetics of congenital
42 hypogonadotropic hypogonadism: peculiarities and phenotype of an oligogenic disease.
43 *Hum Genet* 2020 [Epub ahead of print].
44
45
46
47
48 98. Fraietta R, Zylberstejn DS, Esteves SC. Hypogonadotropic Hypogonadism
49 Revisited. *Clinics* 2013;68(Suppl 1):81.
50
51
52
53
54 99. Fredriks AM, Van Buuren S, Van Heel WJM, Dijkman-Neerincx RHM, Verloove-
55

1 Vanhorick SP, Wit JM. Nationwide age references for sitting height, leg length, and
2 sitting height/height ratio, and their diagnostic value for disproportionate growth
3 disorders. Arch Dis Child. 2005;90(8):807–12.
4
5
6
7
8

- 9 100. Binder G, Schweizer R, Blumenstock G, Braun R. Inhibin B plus LH vs GnRH
10 agonist test for distinguishing constitutional delay of growth and puberty from
11 isolated hypogonadotropic hypogonadism in boys. Clin Endocrinol (Oxf) 2015
12 ;82(1):100–5.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
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NOTES

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TABLES

Table I - Clinical differences between CDGP, GHD and cHH at presentation (2) (3) (15) (16) (17) (18) (25) (26) (29) (30) (33) (44) (67) (68) (97) (99)

	CDGP	GHD	cHH
Initial Height (SDS)	>-2.5	any	>-2.5; 60% of the cases has normal height
Growth velocity	Prepubertal, concordant with bone age	Extremely reduced	Prepubertal
BMI	Reduced	Increased	Normal or increased
Bone Age (BA)	Delayed by 1-3 years	Frequently delayed; possible rare cases of normal/advanced BA for CA if sudden GHD onset occurs or precocious puberty coexists	Delayed
Predicted Adult Height (PAH)	Lower limit of TH	< TH	>TH
Past history illness	Sometimes cryptorchidism (unilateral) or phimosis	Neonatal hypoglycemia, prolonged jaundice, micropenis, midline defects	“Red flags”: cryptorchidism (bilateral), micropenis, hypospadias, renal anomalies, midline defects, hypo/anosmia
Family history	positive for CDGP in 80% of the cases	Parent’s consanguinity; one family member affected if secondary to a specific mutation	One family member affected or presenting hypo/anosmia or neurologic diseases or history of CDGP

Table note:

BMI = body mass index, PAH = predicted adult height, BA = bone age, CA = chronological age, TH =target height.

Table II - Differences of five methods of adult height prediction (PAH) in terms of over-estimation or under-estimation (in cm) compared to actual final height (27) (28) (29) (30) (69)

Method of PAH	boys	girls
Bayley and Pinneau	+3.1 cm	-0.8 cm
Roche-Weiner-Thissen (RWT)	-0.6 cm	+2.3 cm
TH	+1.7 cm	+1.2 cm
Tanner-Whitehouse Mark I (TW-MI)	-7.3 cm	-2.1 cm
Tanner-Whitehouse Mark II (TW-MII)	- 4.2 cm	-1.8 cm

Table III-Final height (FH) in CDGP boys and girls considering their possible treatment with low dose sex steroids (54) (55) (56) (57) (63)

	SDS FH untreated patients	SDS FH treated patients	SDS TH	ΔSDS FH -TH	SDS FH reference population
BOYS					
Crowne et al, EJP, 1991 (n = 43)	-1.6	n. r.	-0.6	-1	n. r.
Poyrazoglu et al, JPEM 2005 (n = 30)	-2.3	n. r.	n. r.	n. r.	+0.12
Couto-Silva et al, JPEM 2005 (n = 70)	-0.7	-1.2	n. r.	n. r.	n. r.
Zucchini et al, EJP 2008 (n = 29)	-1.02	-1.39	-1.12 untreated patients; -1.45 treated patients	+0.1 untreated patients; +0.43 treated patients	n. r.
GIRLS					
Crowne et al, EJP, 1991 (n = 15)	-1.5	n. r.	-0.8	-0.7	n. r.
Zucchini et al, EJP 2008 (n = 16)	-0.78	n. r.	-0.88	+0.1	n. r.

Table note: n.r. = not reported

Table IV - Final height (FH) in GHD boys and girls treated with rhGH at substitutive doses. (86)
(87) (88) (89) (90)

	FH SDS	Δ SDS FH -IH	Δ SDS FH -TH
Reiter E.O. et al (n = 1258)	-0.8 (♂); -1(♀)	n.r.	From -0.6 to +0.2
Cutfield et al (n = 369)	-1.5	+1.7	-0.5
Genentech American group (n = 121)	-0.7 $\pm$ 1.2	n.r.	n.r.
Pozzobon et al (n = 94)	-1.11	From +1.85 to +1.39	From +0.11 $\pm$ 0.80 to +0.26 $\pm$ 0.82

Table note: n.r. = not reported

TITLES OF FIGURES

Figure 1. - Genetic bases of CDGP, GHD and cHH and shared causes.

Figure 2. - Red Flags for cHH. Note that most of these features are shared with GHD and, in a minority of cases, with CDGP.

Figure legend:

ONH = Optic nerve hypoplasia

SOD = Septo optic dysplasia

HPE = holoprosencephaly

EPPG = ectopic posterior pituitary gland

PSIS = pituitary stalk interruption syndrome

MPHD = multiple pituitary hormonal deficiencies

CHARGE = Coloboma, Heart defects, Atresia of choanae, Retardation of growth, Ear abnormalities

Figure 3. - Diagnostic algorithm for the differential diagnosis between CDGP, GHD and cHH

Figure legend

- GHST = growth hormone stimulation test; Criteria for GHST : $SDS\ H < -3$ or $\Delta SDS\ TH-IH < 1.5$ & $GV < 1$
- LDSS = low dose sex steroids; for example testosterone enanthate im 25-50 mg/monthly for boys for 6 months or oestradiol patches 12.5 µg twice weekly for girls for 6 months

**Example of priming protocol before GHST: Testosterone enanthate 100 mg im 7 days before the test for boys; E2 Valerate 1-2 mg orally (if < 20 kg or > 20 kg respectively) 3 days before the test for girls.*

*** Supportive endocrine markers (for boys):*

- Inhibin B < 35 ng/L for Tanner stage 1; < 65 ng/L for Tanner stage 2
- AMH < 20 ng/mL
- LH peak under GnRH test or GnRHa < 4-5 UI/L
- Testosterone levels < 3.6 nmol/L after 3 days or < 9.5 nmol/L after 19 days of hCG test