Advances in differential diagnosis and management of growth hormone deficiency in children

Camille Hage[1], Hoong-Wei Gan[2,3], Anastasia Ibba[4], Giuseppa Patti[5,6], Mehul Dattani[2,3], Sandro Loche[4], Mohamad Maghnie[5,6] and Roberto Salvatori[1] ✉

Abstract | Growth hormone (GH) deficiency (GHD) in children is defined as impaired production of GH by the pituitary gland that results in growth failure. This disease might be congenital or acquired, and occurs in isolation or in the setting of multiple pituitary hormone deficiency. Isolated GHD has an estimated prevalence of 1 patient per 4000–10,000 live births and can be due to multiple causes, some of which are yet to be determined. Establishing the correct diagnosis remains key in children with short stature, as initiating treatment with recombinant human GH can help them attain their genetically determined adult height. During the past two decades, our understanding of the benefits of continuing GH therapy throughout the transition period from childhood to adulthood has increased. Improvements in transitional care will help alleviate the consequent physical and psychological problems that can arise from adult GHD, although the consequences of a lack of hormone replacement are less severe in adults than in children. In this Review, we discuss the differential diagnosis in children with GHD, including details of clinical presentation, neuroimaging and genetic testing. Furthermore, we highlight advances and issues in the management of GHD, including details of transitional care.

The anterior pituitary gland arises from Rathke's pouch by the fourth to fifth week of gestation. At 8 weeks, the growth hormone (GH)-producing somatotroph cells become evident, with abundant immunoreactive cytoplasmic GH expression[1]. Defects of the transcription factors involved in pituitary cell differentiation, or defects of GH secretion, contribute to a heterogeneous group of diseases with different phenotypes, all characterized by impaired growth due to a variable degree of pituitary deficiency. GH deficiency (GHD) can be congenital (genetic and/or associated with malformation) or acquired (due to tumours, physical trauma, inflammation, brain infections or radiotherapy) (BOX 1; Supplementary Table 1; Supplementary Table 2), isolated or associated with other pituitary hormone deficiencies (such as multiple pituitary hormone deficiency (MPHD))[2], and transient or permanent. Most patients have isolated GHD (IGHD) that is idiopathic.

GH is a 191-amino acid protein that is synthesized, stored and secreted in a pulsatile manner by somatotroph cells. The synthesis and release of GH are under the control of various hormones, including GH-releasing hormone (GHRH), somatostatin, ghrelin, insulin-like growth factor 1 (IGF1), thyroid hormone, gonadal steroids and glucocorticoids. Concentrations of GH are higher in the fetal, neonatal and pubertal periods than in adulthood, and increase with chronic malnutrition, exercise, physical trauma and sepsis[1]. In children and adolescents, GH has a role in increasing bone length and density; however, GH is also important throughout life in increasing muscle mass and regulating lipid and carbohydrate metabolism and body water levels. Of note, GH circulates in a variety of different isoforms and the most abundant 22-kDa isoform best reflects pituitary secretion[3]. Approximately 50% of GH circulates bound to GH-binding protein (GHBP). GHBP has the same amino acid sequence as the extracellular component of the GH receptor (GHR) and its serum concentrations are directly related to the expression level of GHRs. Several tissues, especially liver, bone, adipose tissue and muscle, express GHRs.

GH action is exerted directly on target tissues or indirectly by inducing transcription of IGFs. The binding of GH induces a conformation change of constitutively dimerized GHRs by rotation, with the subsequent activation of a phosphorylation cascade involving the JAK–STAT pathway[4]. STAT proteins then migrate to the nucleus and promote the transcription of various genes, such as those encoding IGF1, IGF2, IGF-binding protein 3 (IGFBP3) and acid-labile subunit (ALS).

✉*e-mail: salvator@jhmi.edu*

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Key points

- Growth hormone (GH) affects growth, body composition, metabolic profile, bone mineral density and quality of life; a secretory defect leads to impaired growth and function, known as GH deficiency (GHD).
- GHD can occur in isolation (isolated GHD, IGHD) or in conjunction with other pituitary hormone deficits (multiple pituitary hormone deficiency, MPHD); GHD might be congenital or acquired.
- GHD is familial in 3–30% of affected patients: in IGHD, the most commonly mutated genes are *GH1* or *GHRHR*, whereas MPHD can be caused by mutations in several pituitary-specific transcription factors.
- Congenital hypothalamic–pituitary abnormalities confirmed via imaging, such as anterior pituitary hypoplasia, pituitary stalk anomalies and ectopic posterior pituitary, are common in both children with moderate to severe IGHD and those with MPHD.
- Recombinant human GH (rhGH), 0.16–0.24 mg/kg per week, is used to treat children with GHD; rhGH is best when initiated upon diagnosis and adjusted by serum concentrations of IGF1, height velocity and bone age.
- Transitional care is the shift from paediatric care to adult treatment that provides full-body maturation, metabolic control and improved quality of life for those at risk of persistent GHD.

The main GH effector is IGF1, a 70-amino acid peptide with the ability to bind insulin receptor; IGF1 is mostly secreted by the liver and circulates bound to specific IGFBPs (IGFBP1–6). The IGF1 and IGFBP3 binary complex binds to the large protein ALS, creating a ternary complex that prolongs the half-life of IGF1 and IGFBP3 in the circulation[4]. Of note, IGFBP3 has many other IGF1-dependent and IGF1-independent actions, including both inhibition and enhancement of IGF1 actions and cell proliferation, survival and migration[5]. Furthermore, in addition to GH, malnutrition, thyroid hormone, oestrogens, androgens, chronic diseases, inflammation (such as in coeliac disease or inflammatory bowel disease) and anorexia nervosa can all influence IGF1–IGFBP-3 action[6].

In this Review, we provide a detailed and up-to-date summary of the evaluation and management of children with GHD. We comprehensively review knowledge in differential diagnosis, including clinical presentation, neuroimaging and genetic testing. We also discuss advances in management, adverse effects associated with GH replacement therapy and transitional care from childhood to adulthood.

Diagnosis

The diagnosis of GHD in children is based on medical history, auxological and biochemical investigation, radiological skeletal maturation assessment and neuroimaging of the pituitary region[7,8]. Genetic analysis is indicated in selected patients.

Clinical presentation

The clinical presentation of GHD varies depending on the age of onset. For example, GHD in neonates can be isolated but often presents as MPHD. Neonates and infants might have non-specific symptoms and signs, such as lethargy and poor weight gain, or more specific life-threatening emergencies[9], including respiratory distress, apnoea, cyanosis, poor feeding, hypotonia, long-term cholestatic jaundice, severe hypoglycaemia with or without seizures, and/or neonatal sepsis. Eye abnormalities or nystagmus can be present in patients with optic chiasm involvement. Furthermore, microphallus might be present in patients with IGHD or patients with associated gonadotropin deficiency. Other physical findings can indicate the presence of GHD. For instance, microphthalmia and single central maxillary incisor can be associated with hypopituitarism in holoprosencephaly, whereas midface hypoplasia and frontal bossing suggest GHD independently from its aetiology[10,11]. Intrauterine growth is generally not affected by GHD, and birthweight and length are usually within normal limits, although might be slightly reduced.

The typical GHD clinical phenotype in childhood is persistent growth failure and short stature associated with frontal bossing, depressed nasal bridge, immature appearance, midfacial hypoplasia, delayed dentition, truncal adiposity and micropenis. However, the most common presentation in adolescents is growth retardation and delayed puberty; facial, axillary and pubic hair are usually lacking[12]. Most cases of IGHD in childhood and adolescence are idiopathic; however, brain tumours, infiltrative conditions such as histiocytosis, and infections of the central nervous system (CNS) should always be considered[13]. Cranial irradiation and brain injuries might cause IGHD or MPHD. Some case reports have described the unexplained phenomenon of normal growth during childhood in the absence of GH[14], particularly in association with craniopharyngioma. Possible explanations include the hyperinsulinaemia and hyperleptinaemia associated with obesity, hyperprolactinaemia and GH variants that are not measured by monoclonal assays and could maintain normal serum concentrations of IGF1.

Similar to IGHD, MPHD is heterogeneous, and can be congenital (genetic, perinatal injuries, malformation, physical trauma or pituitary stalk dysgenesis) or acquired (tumours and or surgery) (BOX 1)[13]. The clinical features vary depending on the type of cells affected. In some cases, a specific phenotype can be associated with a particular genetic mutation (for example, *POU1F1* mutations cause GH, thyroid stimulating hormone (TSH) and prolactin deficiencies). Hormonal deficiencies can become evident at different ages throughout life.

Auxology

In children with suspected GHD (BOX 2), an accurate history includes measured parental heights. Physical examination involves measuring the weight, head

Author addresses

[1]Division of Endocrinology, Diabetes, & Metabolism, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA.
[2]Genetics & Genomic Medicine Research and Teaching Department, University College London Great Ormond Street Hospital Institute of Child Health, London, UK.
[3]Department of Paediatric Endocrinology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK.
[4]Paediatric Endocrine Unit, Paediatric Hospital Microcitemico "A. Cao", AO Brotzu, Cagliari, Italy.
[5]Department of Paediatrics, IRCCS Istituto Giannina Gaslini, Genova, Italy.
[6]Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genova, Genova, Italy.

Ectopic posterior pituitary
A disruption of normal embryogenesis of the posterior pituitary resulting in an incomplete downward extension of the diencephalon (infundibulum).

circumference and standing height, or supine length if <2 years old, via accurate instrumentation. Body proportion, BMI, fontanels, dentition, external genitalia, pubertal status and presence of dysmorphic features should be assessed[7]. Furthermore, height velocity should be determined through serial measurements with a minimum interval of 6 months. Of note, skeletal maturity reflects the child's biological age and provides an important contribution to the diagnostic work-up. GHD is unlikely in patients without considerable bone age delay (18–24 months delayed from chronological age)[8].

Box 1 | **Aetiologies of GHD**

IGHD — genetic causes
- *GH1* mutations (GHD type IA or IB)
- *GH1* mutations (GHD type II with evolving pituitary deficiencies)
- *GH1* Kowarski syndrome (bioinactive GH)
- GHD type III (Supplementary Table 1)
- *GHRHR* mutations (GHD type IV)
- *GHS* mutation or variant
- GH in syndromes (Supplementary Table 1, Supplementary Table 2)
- *RNPC3* mutations

MPHD — genetic causes
- Genes implicated in early development of hypothalamic–pituitary region; for example, *HESX1*, *LHX3* and *LHX4*
- Genes implicated in early development of brain and hypothalamic–pituitary region
  - Holoprosencephaly — several genes; for example, *SHH*, *GLI2* and *FGF8*
  - Septo-optic dysplasia and its spectrum involving eyes; for example, *HESX1* and *OTX2*
  - Midline defects (such as cleft palate, persistence of craniopharyngeal canal or dental agenesis); for example, *EDA* and *WNT10A*
  - Extra-brain malformations; for example, *ARNT2*, *CHD7* and *IGSF1*
  - Overlapping with Kallmann syndrome; for example, *FGF8*, *FGFR1*, *PROKR2*, *PROK2*, *CDH7* and *WDR11*
  - Genes associated with other early development conditions
- Genes implicated in cellular differentiation
- Tumour-inducing genes (for example, *SOX2* and *BRAF*)

MPHD — congenital defects
- Midline brain and pituitary developmental defects
- Pituitary aplasia; ectopic posterior pituitary, anterior pituitary hypoplasia and pituitary stalk abnormalities (agenesis or hypoplasia); empty sella
- Congenital CNS mass (hamartoblastoma or hamartoma), cyst, encephalocele

IGHD or MPHD — acquired
- CNS tumours (craniopharyngioma, germinoma, ependymoma, pituitary adenoma, meningioma, medulloblastoma, glioma, metastatic tumours (rare), Rathke's cleft cyst, arachnoid cyst
- Radiotherapy (cranial irradiation for CNS tumours, other malignancies or BMT)
- TBI (accidental, after neurosurgery or subarachnoid haemorrhage)
- Infections (meningitis, encephalitis, tuberculosis or hypophysitis)
- Autoimmune (hypophysitis, APS or anti-PIT1 antibodies)
- Infiltration (LCH, haemochromatosis, chronic blood transfusions or sarcoidosis)

IGHD or MPHD — other causes
- Idiopathic permanent
- Idiopathic transitory

APS, autoimmune polyglandular syndrome; BMT, bone marrow transplantation; CNS, central nervous system; GH, growth hormone; GHD, growth hormone deficiency; IGHD, isolated growth hormone deficiency; LCH, Langerhans cell histiocytosis; MPHD, multiple pituitary hormone deficiency; TBI, traumatic brain injury.

Laboratory investigation

GH thresholds. The clinical suspicion of neonatal GHD can be confirmed by a single GH measurement, preferably obtained during a hypoglycaemic episode, from plasma, serum or newborn blood screening cards[15] within the first week of life. Hypoglycaemia should be confirmed in plasma after rapid sample processing, as the glucose concentration decreases over time. A GH cut-off level that diagnoses GHD in infants has yet to be established[15–17]. Twenty years ago, a random GH measurement of <20 µg/l suggested GHD in the newborn[8], whereas in 2020, Binder and colleagues[15] reported that GH <7 µg/l in the term newborn blood screening card confirms severe GHD with high accuracy. Most guidelines[16] suggest a 5 µg/l cut-off in neonates with additional pituitary hormone deficiencies, or with the triad of ectopic posterior pituitary, anterior pituitary hypoplasia and abnormal pituitary stalk. The specificity of a single GH measurement during spontaneous hypoglycaemia has been questioned; however, normal GH concentration can be useful to exclude GHD[18]. Simultaneous evaluation of cortisol and thyroid hormone concentrations is also recommended. In the case of confirmed biochemical IGHD or MPHD, brain MRI should be obtained (discussed later).

GH stimulation testing. In infancy and childhood, in the absence of signs and symptoms indicative of GHD (BOX 2), other causes of short stature should be ruled out. GH stimulation tests might be required to assess GH secretory capacity. A diagnosis of GHD without GH provocative testing is suggested only in patients who satisfy all the following criteria: auxological characteristics, presence of hypothalamic–pituitary defects on neuroimaging (congenital or acquired) and one additional pituitary hormone deficiency[16].

Many stimulation tests to evaluate GH secretion exist[7,8,19–21]. Clonidine, glucagon, arginine and the insulin tolerance test are the most routinely used. The insulin tolerance test is considered the gold standard and is used to assess GH secretion in response to hypoglycaemia. However, interpretation of the test result is challenging due to an abundance of false-positive results, thereby indicating low specificity and poor reproducibility[22,23]. Albeit less frequently, false-negative results are observed[11]. These issues are due to several factors; for example, the stimuli are not physiological and do not replicate normal secretory dynamics and the periodic secretion of somatostatin might influence the somatotroph response. Additional factors such as obesity, undernutrition, sex, age and puberty also influence GH secretion[3]. For example, GH responses to stimulation tests decrease with increasing BMI[24].

GH secretion increases during puberty and after the administration of sex steroids[25]. In short peripubertal children with delayed puberty, GH testing might yield abnormal results. The most recent guidelines of the Paediatric Endocrine Society published in 2016 (REF.[16]) recommend the use of sex steroid priming before GH testing in prepubertal male individuals >11 years of age and prepubertal female individuals >10 years of age. Sex steroid priming enhances GH secretion and reduces

the number of false-positive results[26–28]. However, when priming is used, GH secretion might be enhanced in a non-physiological manner and can cause false-negative test results, thereby depriving a child of potentially beneficial replacement therapy[26]. Therefore, priming remains controversial[26] with no consensus among European countries[20,21]. Although the age for priming most commonly ranges from 10 to 13 years in boys and from 8 to 12 years in girls[20], some centres prime children as young as 7 years (boys) and 6 years (girls). Of note, the sex steroid preparation and dose differ between centres, and only 25–50% of children undergoing GH testing are primed[20,21]. The steroid preparation used is mostly oral 17β-oestradiol or stilboestrol[27] for two to seven evenings preceding the test, or 50–100 mg intramuscular testosterone enanthate administered 1 week ahead[16].

Owing to poor accuracy, confirmation of a GHD diagnosis requires two failed tests. The provocative tests should be performed after an overnight fast using a standardized protocol under the supervision of an expert team, preferably on two different days. A peak GH concentration below 7 μg/l has been suggested[16]. However, the diagnostic GH peak cut-off is still a matter of discussion and ranges from 5 μg/l to 10 μg/l[7,8,20,23,28–30].

Assay discrepancies across different laboratories contribute to the variability in GH test results. This variability can be reduced if a common pure standard preparation is used for calibration[28]. As suggested by guidelines[11,16,28,31], the best assays should measure the 22 kDa isoform, as it most accurately reflects pituitary GH secretion. Over the past decades, GH assays have changed considerably from non-specific radioimmunoassays to highly sensitive chemiluminescence immunoassays. Although the older assays recognized a spectrum of different GH isoforms together with their homodimers, heterodimers and multimers, the new monoclonal antibodies recognize a precise epitope, picking a narrow spectrum of circulating GH molecules. This advance could partly explain the progressively lower GH concentrations obtained during GH stimulation testing over the past 20 years[3].

Other important biochemical parameters. The interpretation of GH provocative test results should consider all the above aspects as well as other biochemical parameters such as IGF1 and IGFBP3, which are positively correlated with GH secretion[2]. Their serum concentrations show little circadian variation. By contrast GH is secreted in a pulsatile fashion, so a single IGF1 and IGFBP3 measurement is more reliable than a single GH measurement. For these reasons both IGF1 and IGFBP3 have been investigated as alternatives to GH stimulation testing[32–35] and proposed as markers of GH treatment[36]. Of note, IGF1 and IGFBP3 concentrations are influenced by the type of assay[37,38], nutritional status and the presence of chronic illnesses or organ failure, and should be interpreted with regard to age, sex and pubertal status[6,39]. According to some authors, bone age can be used as a surrogate for pubertal status when interpreting IGF1 concentrations; this parameter is particularly relevant for individuals in the peripubertal age group, who have a greater probability of having constitutional delay than having IGHD[40,41].

Several studies have addressed the accuracy of IGF1 and IGFBP3 in the diagnosis of GHD. Most[20,30,33,36] have shown that IGF1 has a good or moderate specificity but low sensitivity in diagnosing GHD, meaning that low IGF1 values with a standard deviation score (SDS) of −2.0 or less are highly predictive of GHD, and values with SDS >0.0 modified by age, sex and pubertal maturation make GHD highly unlikely[28,42,43]. Serum concentration of IGF1 has been reported to of be particularly poor sensitivity in diagnosing GHD in children who have undergone cranial irradiation[44]. In young children, IGFBP3 measurement, which usually offers no advantages over IGF1, might provide additional information as it correlates well with integrated GH secretion and might be more sensitive than IGF1 in the diagnosis of GHD[3,6,19].

Measurement of ALS is not routinely performed as it adds no information to the GH stimulation test, or IGF1 and IGFBP3 measurements. ALS measurement is only indicated when ALS deficiency (OMIM #615961) is suspected[45].

Overall, the decision to perform a GH stimulation test should therefore be based on the severity of short stature, height velocity, history, physical examination, radiological findings and evaluation of IGF1 and IGFBP3 concentrations[16].

Box 2 | Criteria to initiate immediate investigation for GHD

Height
- 3 SD below the mean
- 1.5 SD below the midparental height
- 2 SD below the mean and a height velocity per year that is 1 SD below the mean for chronological age

Height velocity
- 2 SD below the mean over 1 year
- 1.5 SD below the mean sustained over 2 years

Other signs
- Intracranial lesion
- MPHD
- Neonatal GHD

GHD, growth hormone deficiency; MPHD, multiple pituitary hormone deficiency; SD, standard deviation.

Genetic diagnosis of GHD

A genetic origin should be considered in the presence of parental consanguinity, positive family history, craniofacial or brain midline abnormalities or other syndromic features suggestive of a genetic aetiology. Diagnosis of the underlying genetic disorder in congenital GHD is not always straightforward, as current knowledge of the genes implicated in pituitary development remains incomplete, and >80% of patients with MPHD have no genetic diagnosis[2,46]. In addition, determination of pathogenicity of individual genetic mutations in IGHD and/or MPHD can be challenging, as in most patients the disease is probably caused by digenic, oligogenic, epigenetic and/or environmental factors[2]. Next-generation sequencing technologies (whole-exome sequencing

and whole-genome sequencing) might enable more rapid analysis of multiple genes compared with the more laborious candidate gene approach using Sanger sequencing. Whole-exome sequencing might be limited by incomplete coverage, and both whole-exome and whole-genome sequencing can bring problems of data overload, which require refined bioinformatic analyses. As such, the candidate gene approach can still prove useful in situations where extra-pituitary features might point to a specific underlying diagnosis.

Isolated GH deficiency

IGHD is the commonest form of congenital hypopituitarism, with an incidence of 1 in 4,000 to 10,000 live births, of which 3–30% are familial[47,48]. IGHD is inherited in an autosomal recessive (types IA, IB, IV and V), autosomal dominant (type II) or X-linked recessive (type III) manner, usually due to mutations in the genes encoding GH (*GH1*) and the GHRH receptor (*GHRHR*) (BOX 1; Supplementary Table 1)[49]. Of note, IGHD can also arise due to dominant or recessive mutations in developmental transcription factors that influence somatotroph development as part of the normal development of the anterior pituitary (*HESX1*, *SOX3*, *OTX2*, *PROP1* or *POU1F1*)[49]. In this latter scenario, GHD is often the initial presentation before the evolution of subsequent multiple pituitary hormone deficiencies, although GHD might remain as the only endocrinopathy.

GH1 mutations. The *GH1* gene (17q22-24) consists of five exons and is translated into three protein products by alternative splicing, with molecular weights of 22 kDa (191 amino acids, 75% abundance relative to other isoforms), 20 kDa (176 amino acids, 5–10%), and 17.5 kDa (151 amino acids, 1–5%)[49,50]. The 20 kDa and 22 kDa isoforms are biologically active. The severity of IGHD correlates with the deleteriousness of a given mutation. For example, homozygous *GH1* deletions result in type IA IGHD and early, severe growth failure (height less than −4.5 SDS), undetectable GH concentrations and tachyphylaxis to GH treatment due to the formation of anti-GH antibodies in most, but not all, patients[51–53]. Type IA IGHD can also result from severe truncation of the GH molecule secondary to other homozygous or compound heterozygous mutations[45,54,55]. By contrast, patients with type IB IGHD have low but detectable GH concentrations and a persistent response to treatment[49].

The commonest form of genetic IGHD, type II IGHD, is also the most variable in terms of age at presentation and degree of growth failure, with some carriers achieving a height within the normal range[55,56]. This form is caused by splice site or missense mutations in *GH1* that result in low, detectable GH concentrations and occasional anterior pituitary hypoplasia[57,58]. Patients with type II IGHD can develop other pituitary hormone deficits, due to a dominant-negative effect of the 17.5 kDa GH isoform on bioactive 22 kDa isoform production[59,60]. This effect results in protein misfolding and ultimately in impairment of secretory pathways for other pituitary hormones (adrenocorticotropic hormone (ACTH), TSH or luteinizing hormone (LH)). Type II IGHD can also arise from the generation of bioinactive GH, which either fails to activate the GH receptor or results in reduced downstream gene transcription[61,62].

GHRHR mutations. Homozygous or compound heterozygous *GHRHR* mutations cause type IV IGHD, classically presenting with severe growth failure, extremely low GH concentrations that are poorly responsive to stimulation, low concentrations of IGF1 and IGFBP3, and good response to GH replacement therapy[63,64]. Midfacial hypoplasia, neonatal hypoglycaemia and microphallus are less common than in type IA IGHD, although anterior pituitary hypoplasia is very common due to the trophic effect of GHRH on somatotroph proliferation[49,65]. Compound homozygous *GHRHR* mutations (such as c.11G>A and c.236C>T, (p.Arg4Gln and p.Pro79Leu, respectively)) have additionally been described in association with a mild phenotype (untreated near-adult female height of 144 cm, −3.0 SDS) or presentation in mid-childhood (6–8.5 years of age)[66].

Other molecular mechanisms associated with IGHD. The GH secretagogue receptor regulates GH release via its endogenous ligand, ghrelin[67]. Both autosomal dominant and recessive mutations in this receptor have been reported, resulting in a phenotype that ranges from normal GH secretion to partial IGHD, possibly due to a loss in constitutive receptor activity[68,69].

Recessive mutations in *RNPC3*, which encodes a specific protein component of the minor spliceosome, have also been described in association with IGHD type V. The phenotype includes severe postnatal growth retardation, undetectable GH concentrations even on stimulation, undetectable IGF1 and IGFBP3, low–normal prolactin concentrations and anterior pituitary hypoplasia[70]. A 2020 study found the presence of compound heterozygosity for two variants in *RNPC3*, namely c.443G>C, p.[Gly148Ala] and c.259C>T, p.[Gln87*], in three siblings from an African Caribbean family[71]. The phenotype included the presence of other pituitary hormone deficiencies: TSH and prolactin deficiency with hypogonadism, although no gonadotrophin data were presented.

Multiple pituitary hormone deficiency

MPHD is defined as the presence of two or more pituitary hormone deficits and can be syndromic or non-syndromic (BOX 1; Supplementary Table 2). MPHD can present in the neonatal period or later in life. Syndromic MPHD refers to the association of pituitary hormone deficiencies with abnormalities in other structures that share a common embryological origin such as the eyes, midline structures or forebrain. The number of known syndromic MPHD-associated genes continues to increase; however, in most patients a genetic defect still cannot be identified.

Non-syndromic MPHD. Some studies have found that in up to 50% of individuals with familial MPHD, the deficiency is caused by recessive mutations in *PROP1*; the most common mutation is a deletion in exon 2 that leads to protein truncation[72,73]. *PROP1* expression triggers downstream expression of *POU1F1*, which induces terminal differentiation of somatrotrophs, thyrotrophs

and lactotrophs. In addition, *PROP1* expression determines the cell lineages that secrete LH and FSH[74]. As such mutations in *PROP1* are associated with GH, TSH, prolactin, LH and FSH deficiencies, however, patients with such mutations also show a generally late onset of ACTH deficiency but the underlying mechanism is unclear. Of note, the timing of hormonal deficiencies can vary even in patients carrying identical mutations, and importantly, deficiencies can evolve over time. Mutations in *PROP1* can also cause apparent pituitary masses that wax and wane over time, ultimately leading to anterior pituitary involution[75,76].

The second most common form of familial MPHD (25%) is caused by mutations in *POU1F1*, which are associated with GH, TSH and prolactin deficiencies[77]. Most mutations are recessive; however, a frequently occurring heterozygous mutation (p.R271W) has also been identified, where the protein product acts in a dominant-negative manner and inhibits transcriptional activity of the wild-type protein[77,78]. *POU1F1* mutations have largely been reported in a predominantly expressed α-isoform. However, since 2018, studies have also shown mutations in a minor alternatively spliced β-isoform of *POU1F1* that are associated with IGHD, while TSH deficiency can be early or develop much later[79,80].

Mutations in genes such as *ROBO1*, *FOXA2*, *CDON* and *GPR161* have been associated with pituitary stalk interruption syndrome (PSIS, discussed later) and MPHD. Mutations in *CDON* are associated with non-syndromic MPHD[81], whereas mutations in *ROBO1*, *FOXA2* and *GPR161* are associated with other extra-pituitary clinical features[82–84].

Syndromic MPHD. One form of syndromic MPHD is septo-optic dysplasia (SOD), which is defined by the presence of at least two of the triad of optic nerve hypoplasia, midline forebrain defects and pituitary hypoplasia, or hypopituitarism[85]. Of patients with SOD, 30% have all three features and 62% have hypopituitarism[86]. Neuroradiological abnormalities can include anterior pituitary hypoplasia, or an ectopic posterior pituitary or an absent infundibulum, all predictors of hypopituitarism[87]. Mutations in genes encoding transcription factors involved in early pituitary development such as *HESX1* (homozygous and heterozygous) and *TCF7L1* (heterozygous) have been found in some patients with SOD[88,89]. However, its aetiology remains multifactorial, with other environmental factors (such as viral infections, vascular changes, alcohol or drug exposure) being possibly implicated, with incidence being higher in children born to younger mothers than in children born to older mothers[90]. Of note, a 2020 study suggested considerable differences between patients with SOD and patients with MPHD without associated midline abnormalities, in terms of the timing and nature of endocrinopathies, and the likelihood of spontaneous puberty[91].

The coexistence of MPHD with ocular abnormalities, such as anophthalmia or bilateral microphthalmia, suggests the presence of genetic mutations in *SOX2*, *OTX2* or *RAX*. In *SOX2* or *OTX2*, only autosomal dominant mutations have been described. The classic presentation of *SOX2* loss-of-function mutations is hypogonadotrophic hypogonadism and variable GH deficiency; however, these mutations can also be associated with other abnormalities including spastic diplegia, epilepsy, oesophageal atresia and/or tracheoesophageal fistula, hypothalamic hamartoma, hippocampal hypoplasia, ventriculomegaly, absent septum pellucidum, corpus callosum agenesis, sensorineural hearing loss and male genital tract abnormalities[92–94]. By contrast, patients with *OTX2* mutations can present with IGHD or MPHD, but these mutations might also be associated with retinal degeneration, ectopic posterior pituitary or even completely normal eye development[95,96]. In 2019, compound heterozygous and homozygous mutations in *RAX* were reported in association with anophthalmia, MPHD with central diabetes insipidus, and cleft lip and palate[97].

X-linked mutations in *SOX3* have been reported in association with type III IGHD or MPHD and anterior pituitary hypoplasia. Other more variable features of these mutations include mental retardation or developmental delay, posterior pituitary ectopy, or the presence of a persistent craniopharyngeal canal[98–100]. Of note, mutations in *OTX2* and *SOX2* can also be associated with developmental delay.

Mutations in the LIM family of homeobox genes *LHX3* (homozygous and compound heterozygous) and *LHX4* (homozygous and heterozygous) have been reported in MPHD. Mutations in these genes can be associated with a normal, hypoplastic or even enlarged pituitary gland[101]. *LHX3* mutations are linked with a short neck with limited rotation, spinal abnormalities and sensorineural hearing loss[102,103]. By contrast, in *LHX4* mutations, the neck and hearing are normal, but other features can include an ectopic posterior pituitary, hypoplastic corpus callosum and Chiari malformation[101]. Homozygous *LHX4* mutations are associated with early neonatal death and severe panhypopituitarism[104]. In 2015, homozygous loss-of-function mutations in *PNPLA6* (the causal gene responsible for Oliver–McFarlane and Laurence–Moon syndromes) were found to be associated with progressive cerebellar ataxia or atrophy, chorioretinal dystrophy, and variable hypopituitarism that ranged from GH and TSH deficiencies to normosmic hypogonadotrophic hypogonadism[105].

Of note, in holoprosencephaly, central diabetes insipidus is the most common form of hypopituitarism. However, holoprosencephaly with MPHD or panhypopituitarism has been associated with mutations in *GLI2*, *FGF8* and *TGIF1* (REFS[106–108]). The list of genetic syndromes associated with GH deficiency is rapidly expanding and includes mutations in *BMP4*, *PITX2*, *ARNT2*, *EIF2S3*, *FOXA2*, the ciliopathy gene *IFT172*, the channelopathy gene *KCNQ1*, *ROBO1*, *GPR161*, *TBC1D32* and *GLI3* (REFS[2,109]). Many of these genes (*BMP4*, *GPR161*, *EIF2S3*, *IFT172* and *KCNQ1*) are also implicated in early hypothalamic–pituitary development[2,109]. Several genes associated with Kallmann syndrome (*ANOS1*, *FGFR1*, *PROKR2*, *CHD7* and *WDR11*) have also been described in association with GH deficiency, MPHD and SOD[2,110,111]. Finally, mutations in genes more predominantly associated with other forms of hypopituitarism such as *IGSF1* (central hypothyroidism) and

Holoprosencephaly
A syndrome caused by failure of separation of the cerebral hemispheres and ventricles and associated with a wide range of midline facial defects, ranging from cyclopia to midfacial hypoplasia, cleft lip and/or palate and a single incisor.

T2-DRIVE
A T2-weighted driven equilibrium (DRIVE) imaging obtained via turbo fast spin-echo sequences at sub-millimetre thickness, which provide excellent contrast between the cerebrospinal fluid and the adjacent parenchymal structures.

PCSK1 (ACTH deficiency) can also be associated with GH deficiency and MPHD[112,113].

Pituitary stalk interruption syndrome. PSIS is a rare spectrum of congenital abnormalities of the pituitary gland including: an absent or ectopic posterior pituitary; thin, hypoplastic or interrupted pituitary stalk; with or without hypoplasia or aplasia of the anterior pituitary gland[108,114–120]. The syndrome is more common in boys, has a variable age at diagnosis and also occurs sporadically in the majority of patients[114–119]. Recombinant GH post-marketing surveillance databases suggest that around 4–8% of patients with GHD have PSIS[117–120].

Only 5% of patients with PSIS have identifiable genetic mutations, and several genes that overlap with other causes of GHD and MPHD (for example, *CDON*, *HESX1*, *OTX2*, over-dosage and under-dosage of *SOX3*, *LHX4*, *GLI2*, *TGIF1*, *FOXA2*, *IFT172*, *ROBO1*, *GPR161* and *TBC1D32*) have been associated with ectopic posterior pituitary. An association also exists between PSIS and other midline defects[102,115,116,118,121]. Digenic inheritance (for example, *PROKR2* and *WDR11* (REF.[122])) has also been reported. Furthermore, an association can occur between PSIS and extra-pituitary abnormalities such as biliary ciliopathy with homozygous *TTC26* mutations[123] and Fanconi anaemia[119,124,125]. However, like SOD, a polygenic and multifactorial aetiology is probable, and, in one study, up to 83% of patients with sporadic PSIS had multiple heterozygous variations in genes largely affecting Notch, Shh and Wnt signalling[124]. More recent whole-exome studies from 2018 and 2020 identified further candidate genes (for example, *FAT2*, *DCHS1*, *DCHS2*, *ROBO2*, *CCDC88C*, KIF14 and *KAT6A*)[126,127].

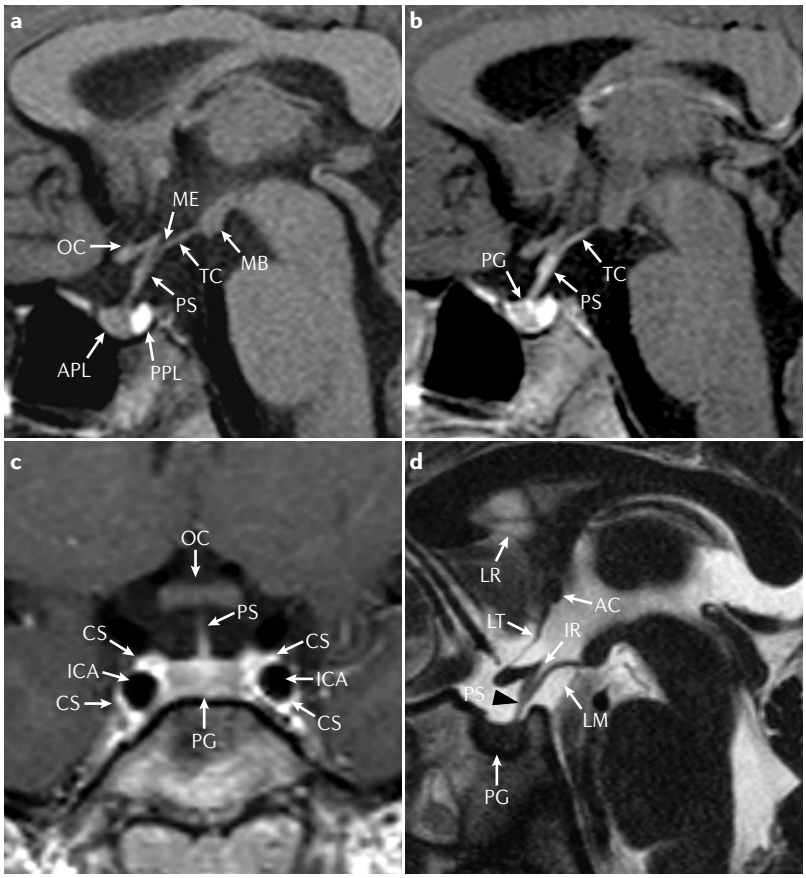

Fig. 1 | **Normal MRI study in a healthy 9-year-old boy.** MRI protocol consisted of 2–3 mm thick, high-resolution spin-echo T1-weighted and turbo fast spin-echo T2-weighted images in the sagittal and coronal planes. The T2-DRIVE sequence was acquired in the sagittal plane with a slice thickness of 0.6 mm (25 slices) and a scan time of 2 min and 32 s, using a 3D technique with isotropic voxels (0.6 × 0.6 × 0.6 mm) that allows multiplanar reformatting with no geometric distortion. **a** | A sagittal T1-weighted image of the hyperintense posterior pituitary lobe (PPL), anterior pituitary lobe (APL), pituitary stalk (PS), median eminence (ME), optic chiasm (OC), mamillary body (MB) and tuber cinereum (TC) (white arrows). **b** | A gadolinium-enhanced sagittal T1-weighted image of the pituitary gland (PG), PS and TC (white arrows). **c** | A gadolinium-enhanced coronal T1-weighted image of the internal carotid arteries (ICA) and cavernous sinuses (CS) (white arrows); the PG cannot be confidently separated into the APL and PPL. **d** | A sagittal T2-DRIVE image, in which PS (black arrowhead) is optimally depicted with sharp delineation of the infundibular recess of the third ventricle (IR); additional midline structures shown include the lamina rostralis (LR), anterior commissure (AC), lamina terminalis (LT) and Liliequist membrane (LM).

Neuroimaging in hypopituitarism

The diagnostic accuracy of MRI has led to an enormous increase in our knowledge of pituitary morphology and function, which has improved the differential diagnosis of hypopituitarism[114,119]. MRI has also improved the early identification of neuroimaging hallmarks of evolving anterior pituitary hormone deficiencies, the prediction of long-term outcomes and has aided genetic counselling. Brain MRI should be performed in children with GHD to avoid missing hypothalamic–pituitary abnormalities or tumours[85]. Equally, MRI of the hypothalamic–pituitary region in neonates or infants with hypoglycaemia and symptoms that suggest congenital hypopituitarism, during the neonatal and postnatal period, is valuable in identifying midline defects and pituitary abnormalities[7].

MRI protocol in hypopituitarism

The correct interpretation of MRI scans requires detailed knowledge of the normal features of the pituitary gland and of its changes within the same individual over time (Supplementary Table 3)[128,129]. The assessment includes the evaluation of signal intensity, shape, size, position of the anterior pituitary, posterior pituitary and pituitary stalk, and connection with surrounding tissues (FIG. 1). In addition to high-resolution sellar MRI, one or more survey sequences of the entire brain, a fluid attenuation inversion recovery and a diffusion-weighted imaging sequence in the axial plane should be acquired to rule out additional CNS abnormalities; contrast-enhanced imaging can safely be omitted in patients with IGHD, if T2-DRIVE (FIG. 1d) has been performed[130].

MRI findings in hypopituitarism

Patients with idiopathic, congenital or genetically determined GHD can present with one of three different phenotypes: first, with normal or hypoplastic pituitary gland or empty sella, normal or thin pituitary stalk, and normal hypothalamic–pituitary connection with or without CNS abnormalities; second, with anterior pituitary hyperplasia or intermittent hyperplasia or enlarged sella: and third, with moderate to severe hypoplastic pituitary gland or small sella, thin or hypoplastic or absent pituitary stalk with an ectopic posterior pituitary (sometimes

Box 3 | **MRI findings in hypopituitarism**

Idiopathic GHD

Normal pituitary
No evidence of morphologic, volumetric or signal abnormalities.

Isolated pituitary hypoplasia
Small anterior pituitary (height <3 mm) or severe hypoplasia (height <2 SDS) housed within a small or normal pituitary fossa.

Empty sella or intraseller arachnoidocele
Deep and small or enlarged pituitary fossa, mainly filled with CSF. The anterior pituitary appears as a thin layer along its floor. Laminar appearance of the posterior lobe flattened against the dorsum sellae. Stretched pituitary stalk, posteriorly dislocated.

Pituitary gland agenesis or atrophy
Absence of a clearly identifiable pituitary gland. Small and flat sella.

Ectopic posterior pituitary
Variable degree of anterior pituitary hypoplasia, absence or marked thinning or hypoplasia of the pituitary stalk and ectopic posterior lobe from the median eminence to the distal stalk. Sometimes double or partial ectopic posterior pituitary or ectopic posterior pituitary flattened within a thin pituitary stalk.

Central nervous system abnormalities
Chiari I malformation; sporadic non-complex abnormalities.

Genetic GHD

Normal pituitary
No evidence of morphologic, volumetric and signal abnormalities.

Isolated pituitary hypoplasia
Small anterior pituitary (height <3 mm) or severe hypoplasia (height less than −2 SDS) housed within a small or normal pituitary fossa.

Empty sella or intraseller arachnoidocele
Deep and small or enlarged pituitary fossa, mainly filled with CSF. The anterior pituitary appears as a thin layer along its floor. Laminar appearance of the posterior lobe flattened against the dorsum sellae. Stretched pituitary stalk, posteriorly dislocated.

Pituitary gland agenesis or atrophy
Absence of a clearly identifiable pituitary gland. Small and flat sella.

Anterior pituitary hyperplasia
Anterior pituitary enlargement mimicking a sellar mass lesion (associated with *LHX3*, *PROP1* or *SOX2* mutations). Tendency to spontaneous regression and evolution into pituitary hypoplasia or intermittent hyperplasia in *PROP1*-associated GHD; cystic pituitary in *LHX3*-associated GHD.

Ectopic posterior pituitary
Variable degree of anterior pituitary hypoplasia, absence or marked thinning or hypoplasia of the pituitary stalk and ectopic posterior lobe from the median eminence to the distal stalk. Sometimes double or partial ectopic posterior pituitary or ectopic posterior pituitary flattened within a thin pituitary stalk.

Central nervous system abnormalities
Persistent craniopharyngeal canal, Chiari type I, Chiari type II, corpus callosum dysgenesis, septum pellucidum agenesis, vermis cerebellar dysplasia, periventricular heterotopia, basilar impression, sellar or suprasellar arachnoid cyst, tentorial anomaly, cortical dysplasia, schizencephaly, frontotemporal lobe hypoplasia, holoprosencephaly, hippocampal abnormalities, absence of internal carotid artery, absence or hypoplasia of olfactory bulbs and olfactory tracts, syringomyelia, hypothalamic hamartoma, variable spectrum of abnormalities in septo-optic dysplasia (optic nerve hypoplasia or aplasia, thin optic tracts, coloboma, anophthalmia, microphthalmia, midbrain–hindbrain abnormalities) and other forebrain, midbrain and hindbrain anomalies.

MRI, magnetic resonance imaging; GHD, growth hormone deficiency; SDS, standard deviation score; CSF, cerebrospinal fluid.

double) that is located anywhere from the median eminence to the distal stalk (as seen in PSIS)[114,115,119]. IGHD is more commonly associated with either normal pituitary anatomy or hypoplastic anterior pituitary or empty sella with normal pituitary stalk, whereas PSIS is most frequently associated with MPHD. Rarely, the anterior pituitary can be hyperplastic with normal posterior pituitary location and normal pituitary stalk[114,115,119], whereas congenital absence or agenesis or atrophy of the pituitary gland is very uncommon[9,116] (BOX 3).

Hypopituitarism with normal pituitary stalk. Pituitary hypoplasia is defined as a small anterior pituitary housed within a small or normal pituitary fossa, and can either be isolated or might occur as a part of complex malformative syndromes including SOD and/or forebrain, midbrain and hindbrain abnormalities[131]. Previous studies in children with hypopituitarism have found a prevalence of normal pituitary of 1–44% or anterior pituitary hypoplasia of 19–84%[119]. These findings vary among studies, but two large studies in more than 13,000 and 8,000 children, showed that 80–86% have normal pituitary gland anatomy whereas 4–9% have hypoplasia[117,120].

The inappropriate use of anterior pituitary hypoplasia as a synonym for partial or total empty sella is worth mentioning. In essence, empty sella (also called intrasellar arachnoidocele) indicates an intrasellar herniation of the subarachnoid spaces through an incompetent sellar diaphragm (arachnoid diverticulum), where the pituitary gland narrows or flattens with consequent enlargement of the pituitary fossa[132]. In addition, the posterior lobe is flattened against the dorsum sellae and the pituitary stalk appears thin and elongated. Secondary empty sella can develop after surgery, radiotherapy or vascular atrophy. In such cases, it is essentially an 'ex vacuo' phenomenon where the intracranial subarachnoid space secondarily extends into the sella. Empty sella is seldom causally associated with hypopituitarism with a prevalence in children with hypopituitarism of 5–9%, which increases with age[132]. An empty sella is found in about 10% of patients with IGHD[133], and the presence of a small pituitary fossa might help distinguish pituitary hypoplasia from a partially empty sella. MRI findings in patients with genetic forms of IGHD or MPHD are summarized in BOX 3 (REFS[75,76,109,114,116,119,134,135]).

Hypopituitarism with pituitary stalk interruption syndrome. PSIS is characterized by its classic triad as mentioned earlier. However, in the past few decades, PSIS has been widened to include patients with one feature such as ectopic posterior pituitary, or interrupted stalk, or interrupted pituitary stalk with absent posterior pituitary[115,118]. Rarely, double or partial ectopic posterior pituitary can be documented[114–116,119,136] (FIG. 2). PSIS remains a complex aetiology involving several factors including epigenetics, environment, drugs and genetics.

Animal experiments show that pituitary stalk transection results in the formation of an ectopic posterior pituitary and that pituitary stalk ischaemia resulting from perinatal asphyxia or breech delivery is associated with ectopic posterior pituitary[137–140]. These findings suggest that PSIS arises as the result of a triggering perinatal event that causes hypoxia on the background of a genetic predisposition. This congenital hypothesis is first supported by the findings of a study in 1991 (REF.[140]) and subsequently in a large series of patients with PSIS, which suggested a prenatal origin[141].

By contrast, perinatal injury has been found in >80% of patients with hypopituitarism[139,140]. For instance, the increased prevalence of maternal antenatal drug and

alcohol abuse, as well as a lower maternal age in children with SOD, led Lubinsky[142] to suggest that SOD might occur secondary to a prenatal vascular disruption sequence. Yet, a lack of experimental evidence supports the vascular origin. Therefore, the role of prenatal environment or birth trauma remains possible and the worsening of a pre-existing condition due to hypoxia cannot be disregarded. Additionally, pituitary abnormalities might have a role in increasing the risk of breech presentation, based on data showing that breech delivery is five times more common in patients with hypothalamic–pituitary abnormalities associated with MPHD[143,144].

Indeed, after the congenital hypothesis was proposed, subsequent MRI findings of ectopic posterior pituitary in several patients with GHD carrying genetic mutations[109,115,116,121,145] were largely favourable to a prenatal origin hypothesis. In these studies, the prenatal hypothesis was evidenced by the association of GHD with several midline defects, with the absence of perinatal adverse events in two-thirds of patients, with cephalic delivery in about 50% and caesarean section in 15% of patients, as well as the association with familial cases and mutations in several genes encoding transcription factors involved in embryonic hypothalamic–pituitary developmental processes.

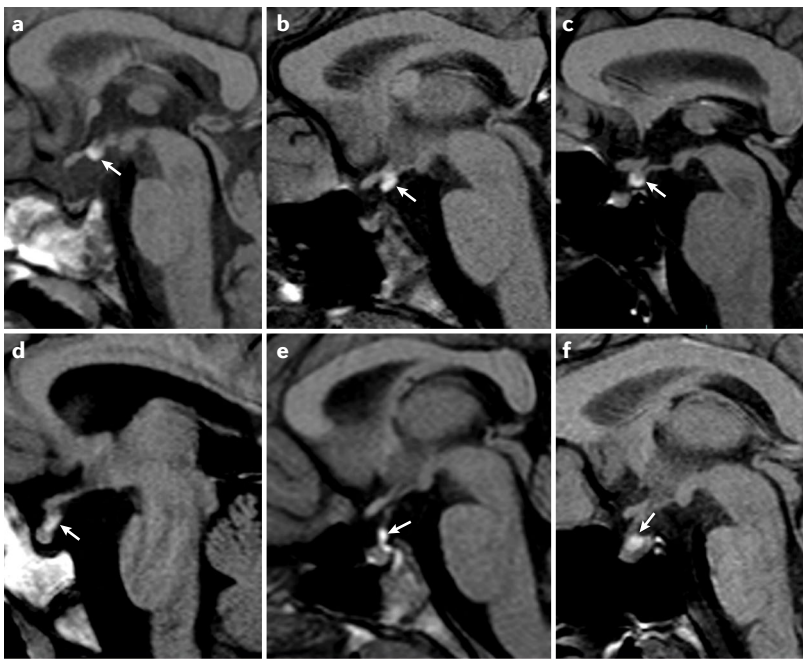

Fig. 2 | **Pathological MRI in children with hypopituitarism.** Sagittal T1-weighted images showing the classic triad of ectopic posterior pituitary (arrows; associated with a variable location), median eminence (parts **a**, **b**), mid-pituitary stalk (parts **c**, **d**) with a double posterior pituitary (part **e**) and distal stalk (parts **e**, **f**). The pituitary stalk is absent (parts **a**, **b**, **c**), or hypoplastic or thin (parts **d**, **e**, **f**). The anterior pituitary is of variable size from severe hypoplasia (parts **a**, **b**) to mild hypoplasia (parts **c**–**f**). Current practice points for MRI work-up in patients with hypopituitarism are as follows. MRI without contrast medium using T2-DRIVE sequences of the hypothalamic–pituitary region and the entire brain (forebrain, midbrain and hindbrain) is highly recommended in neonates, infants and children with signs and symptoms suggestive of hypopituitarism (such as hypoglycaemia, cholestatic jaundice and other signs). First-line MRI examination without growth hormone (GH) testing could be performed. MRI is also highly recommended in children and adolescents with severe short stature and GH testing compatible with a diagnosis of GH deficiency (GHD), in children and adolescents with multiple pituitary hormone deficiency, and in children with isolated GHD (IGHD) and severe short stature (developing pituitary defects are possible over time). MRI could be of low value in children with IGHD and less severe GHD defined based on the local GH cut-off (>3, >5, >7 or >10 ng/ml). A personalized decision is advisable.

Hypothalamic–pituitary MRI anatomy and pituitary function. Several studies have found higher rates of ectopic posterior pituitary in patients with MPHD than in patients with IGHD[114,119,140,143,144,146]. MRI identification of the triad of ectopic posterior pituitary, anterior pituitary hypoplasia and pituitary stalk agenesis is of great value in recognizing patients at risk of developing pituitary hormone deficiencies. In particular, small size and location of ectopic posterior pituitary are predictive of MPHD development[146,147].

By contrast, the presence of a vascular component of the stalk has a positive prognostic value, as patients in whom a pituitary stalk cannot be identified after administration of the contrast agent gadolinium-DTPA have a 27 times greater risk of developing MPHD than those with a residual vascular pituitary stalk[148]. A detailed study of the pituitary stalk with gadolinium-DTPA is no longer recommended in congenital hypopituitarism provided T2-DRIVE has been performed[130]. The pituitary stalk can be better recognized by T2-DRIVE than by conventional T1-weighted and T2-weighted imaging (FIG. 2). This T2-DRIVE observation raises the question about its prognostic value in predicting the deterioration of pituitary defects[130].

The current data suggest that MRI scans can help predict the response of an individual patient to therapy. The relationship between pituitary MRI characteristics and growth response after treatment with recombinant human GH (rhGH) has shown that hypothalamic–pituitary structural abnormalities are key parameters in predicting growth response[149]. In addition, patients with GHD with ectopic posterior pituitary perform better in terms of adult height achieved than those with normal or hypoplastic anterior pituitary on MRI[117,120]. MRI findings in individuals with IGHD and MPHD are summarized in FIG. 3.

Management

Treatment with rhGH

The established treatment for GHD in children is rhGH, also known as somatropin[150]. This aqueous biosynthetic GH is administered subcutaneously at night to follow the GH secretory pattern during sleep[16]. Most pharmaceutical brands, which share a similar effect, have a multiple-dose pen for easier administration. Several sustained-release GH preparations that are administered weekly have been developed since 2007, in order to ease the burden of use[151]. These formulations substantially vary in molecular weight and ionic charge, with some using fusion proteins to affect the access of GH to target tissues[152]. Treatments that require fewer injections might offer increased acceptance, tolerability and flexibility than daily rhGH[153]. Indeed, the lack of adherence to daily rhGH has been hypothesized as the reason why many children remain below the mid-parental target height despite treatment[154]. No significant differences in effectiveness and adverse events have been identified when

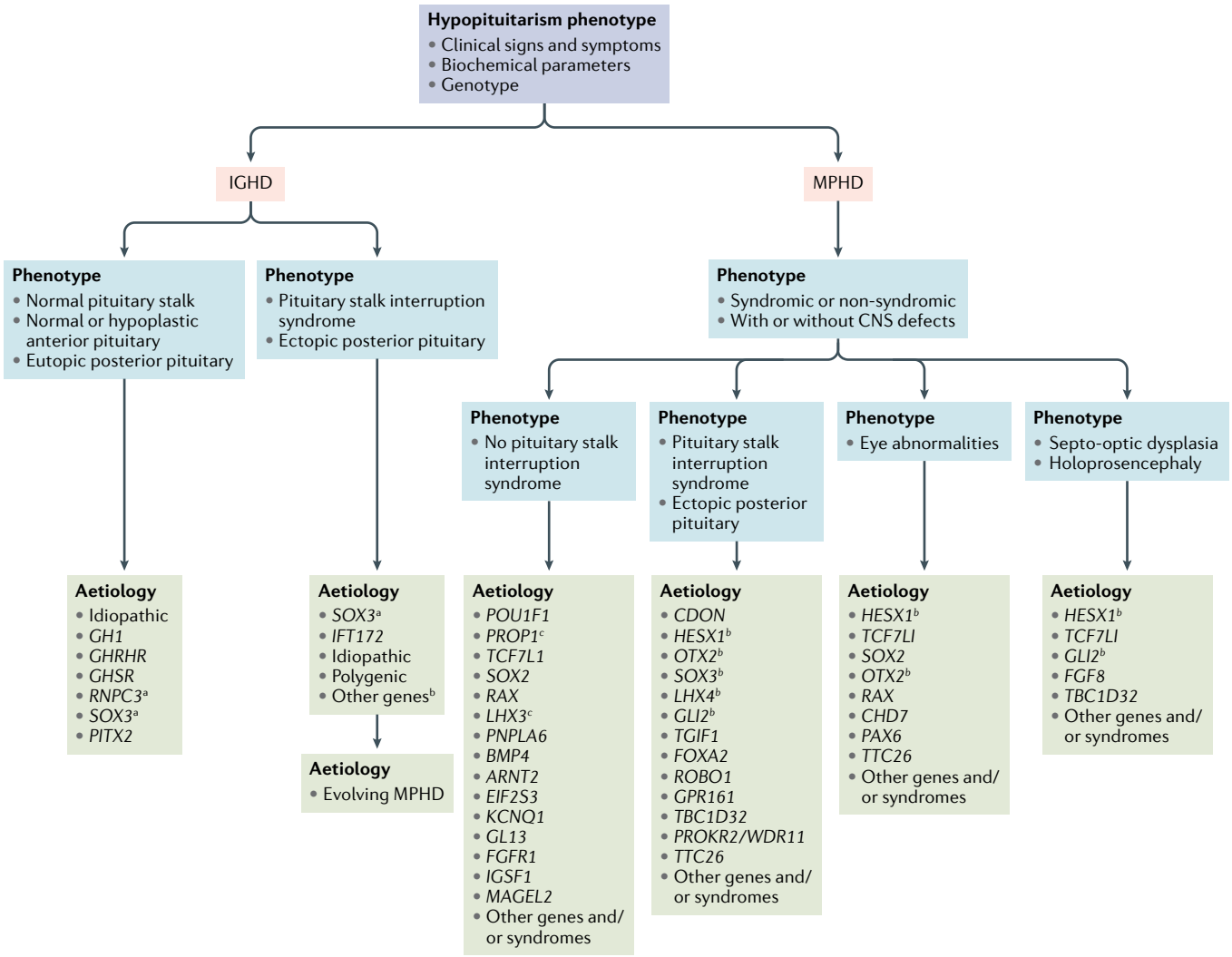

Fig. 3 | **MRI findings in congenital hypopituitarism based on genotype.** A practical algorithm showing MRI assessment of patients with suspected hypopituitarism (grey box shows the initial phenotype). Correlations between MRI phenotype and genotype, based on endocrine status in patients with IGHD, syndromic or non-syndromic MPHD provide a straightforward approach to breaking down the differential diagnosis lists into more manageable categories. Blue boxes show patient phenotype and green boxes show different aetiologies. [a]IGHD or MPHD; [b]Variable MRI pituitary abnormalities including normal pituitary stalk, ectopic posterior pituitary and CNS abnormalities; [c]Anterior pituitary with a variable transient pattern from hyperplasia to hypoplasia and vice versa. CNS, central nervous system; IGHD, isolated growth hormone deficiency; MPHD, multiple pituitary hormone deficiency.

sustained-released GH was compared with daily rhGH in a meta-analysis of clinical trials published between 2012 and 2018 (REF.[155]). Therefore, long-acting preparations might be a promising replacement for daily rhGH, with a few questions still not completely answered, such as the methods of dose adjustment, timing of monitoring of IGF1, safety, efficacy and cost-effectiveness[152]. Some safety concerns revolve around the formation of anti-drug antibodies in patients, as well as efficacy limitations in large preparations of GH fusion proteins due to size disparity with key target tissues, which leads to different metabolic side effects[152]. Hence, post-marketing surveillance will be crucial.

Optimal dosage. Currently, the recommended daily GH dosage based on weight is 0.16–0.24 mg/kg per week (0.022–0.035 mg/kg per day) with a maximum dose that should not exceed 0.3 mg/kg per week[7,16,19]. The dose might be increased at puberty, although this change is not recommended routinely[16]. The medication is best initiated as soon as the diagnosis is confirmed with the optimal outcome occurring while bone growth plates (epiphyses) are open (generally at age <15 years in girls and <17 years in boys)[154]. However, the response varies considerably between individuals according to the diagnostic criteria. Patients with less severe GHD and/or those who start medication at an older age will have a worse response to therapy than younger patients with more severe disease, respectively[156–158]. Peak GH concentrations during stimulation testing, age at onset of therapy and height difference from mid-parental target height are the most important predictors of the first-year height velocity. Although one would hope that using a personalized rhGH dose that considers these factors could lead

to low variability in medication response, studies have questioned the reliability of predictive factors[159].

The method used for dosage refinement has been the subject of much debate[16,159,160]. An approach that is broadly used is to adjust the GH dose based on serum concentrations of IGF1. Although keeping the IGF1 concentration within the age-adjusted normal range is reasonable, no consensus exists on the optimal target level; some studies have found that increased concentrations of IGF1 correlate with increased height gain without adverse effects[19]. Regardless, at the expected first follow-up, a decrease in dosage is recommended if the concentration of IGF1 has increased beyond the normal range, while exploring other possible reasons such as an incorrect diagnosis[16].

Treatment response. The optimal response to therapy is monitored after the first year via height velocity parameters; these are height velocity and/or change in height SDS that both intrinsically correct for age and sex. Although height velocity is easier to compare with height velocity curves and is more routinely used, height SDS helps assess children with height measurements that fall well below the standard percentile[156,160].

Catch up growth depends on the severity of GHD, with children affected by organic pathologies (such as hypothalamic–pituitary damage by lesion, surgery and/or radiation) being more likely to show a more marked growth response than children with more moderate forms of IGHD; however, the peak response during a stimulation test in children with IGHD does not seems to predict the degree of catch up growth[161]. Following a year of GH therapy, the medication response is considered poor if the height SDS improvement is lower than 0.4 (REFS[156,160,162]). The causes behind a low response to therapy include lack of adherence, improper rhGH administration, hypothyroidism, concurrent chronic disease, complete osseous maturation and/or presence of GH antibodies. Some researchers suggest monitoring bone age; however, an issue remains with the interobserver interpretation of radiographic imaging, and possible GH acceleration of bone maturation before imaging is carried out[163,164]. BoneXpert, an automated method for analysis of hand radiographs in children, has been in use to overcome this issue, yet larger studies are needed to validate its accuracy[165–167].

Adverse effects

Although the effectiveness of rhGH therapy is undeniable, multiple potential adverse effects need to be monitored. In the short term, intracranial hypertension with increased intraocular pressure, and slipped capital femoral epiphysis can arise. Benign intracranial hypertension is to be considered in patients with headache, nausea, visual disturbance and dizziness, and should trigger an ophthalmological referral[168]. If confirmed, patients should stop treatment until intracranial pressure is resolved (usually around a month) and then resume at a lower dose. Slipped capital femoral epiphysis and intracranial hypertension are seen more commonly in patients with Turner syndrome, Prader–Willi syndrome, chronic renal insufficiency and organic GHD than in children with IGHD[169]. Childhood cancer survivors who were previously exposed to total body irradiation have a higher risk of slipped capital femoral epiphysis during rhGH therapy than children with other causes of GHD[170]. For patients who develop this complication, an orthopaedic consultation for pinning of the capital femoral epiphysis should be recommended. Additionally, rhGH treatment can induce a progressive worsening of pre-existing scoliosis, which might require orthopaedic intervention. Other rare adverse effects have been reported, such as transient gynaecomastia, increase in growth of non-malignant naevi, carpal tunnel syndrome, arthralgia, oedema, various musculoskeletal comorbidities caused by water and sodium retention, exacerbation of obstructive sleep apnoea due to tonsillar hypertrophy, and pancreatitis. However, the causal relationship between rhGH therapy and these adverse events is yet to be confirmed[171].

Mortality and risk of malignancy. Assessing mortality in patients with GHD remains difficult owing to the underlying comorbidities leading to GHD. The existing evidence does not support a clear association between GH replacement therapy and risk of death, as has been shown in the Safety and Appropriateness of Growth Hormone Treatments in Europe (SAGhE) study[172–174]. This study assembled cohorts of patients treated in childhood with rhGH in eight European countries since 1984 and followed them for cause-specific mortality and cancer incidence. Although the French report noted concerns regarding the safety of rhGH, with a 33% increase in all-cause mortality and a higher risk of death in patients receiving higher doses (>0.05 mg/kg per day) than lower doses, other reports from Netherlands, Belgium and Sweden could not confirm these findings. In the French report, the main causes of mortality were bone tumours and cerebral haemorrhage. The SAGhE study was updated in 2020 with results that showed no significant increase in overall mortality patients with low risk (those with IGHD or idiopathic short stature)[174]. Conversely, patients with increased risk (those with MPHD and/or comorbidities), showed increased mortality due to cardiovascular and haematological causes that was associated with the underlying conditions[175]. Mortality was not associated with mean daily or cumulative rhGH dose[174]. Similar findings in other studies have been reported[175].

An increased risk of malignancy caused by long-term rhGH treatment in children has been hypothesized. This hypothesis is based on the observation that adults without GHD who have concentrations of IGF1 that fall in the upper quartile show an increased risk of breast and prostate cancer, possibly due to the growth-promoting effects of GH[176]. However, no report of an increase in new primary malignancies has been noted in any patients without risk factors (mostly those with idiopathic GHD) treated with rhGH[16]. Thus, cancer monitoring is not recommended in these patients. For childhood cancer survivors, the correlation between rhGH treatment and secondary cancer is controversial. GH therapy does not increase the regrowth risk of pituitary adenomas or craniopharyngiomas[177]. Regardless, in patients with GHD and cancer, waiting for a full year upon completion

Slipped capital femoral epiphysis
A disorder seen in adolescents in which the growth plate is damaged and the femoral head moves ('slips') with respect to the rest of the femur: the head of the femur stays in the cup of the hip joint while the rest of the femur is shifted.

of cancer therapy to confirm its eradication has been suggested before the initiation of rhGH[21].

Effects on metabolism. Monitoring of impaired glucose metabolism and potential diabetes mellitus should be considered in patients at risk, with risk factors including predisposition to diabetes mellitus via positive family history, small for gestational age, the metabolic syndrome or history of gestational diabetes mellitus in their mother[178]. Furthermore, as GH decreases insulin sensitivity, patients diagnosed with diabetes mellitus might have increased insulin requirements. However, GHD might alter glucose metabolism due to impaired body composition (decreased ratio of lean to adipose tissue mass), which GH treatment can reverse. Therefore, rhGH treatment should not be withheld from patients with coexisting diabetes mellitus or a predisposition to diabetes mellitus. Glycaemic control might worsen upon the initiation of rhGH treatment, whereas a beneficial effect on glucose metabolism will only be apparent with time after improvement in body composition[179]. In these patients, starting with low doses of rhGH is recommended. Additionally, rhGH can increase T_4 catabolism via the increase in the peripheral conversion of T_4 to T_3, and cortisol catabolism via the inhibition of 11β-HSD1 in the conversion of cortisone to cortisol, thereby indicating central hypothyroidism or hypoadrenalism. Hence, the adrenal and thyroid axes should be periodically checked after rhGH therapy is started or the dose is increased, especially in those with structural hypothalamic–pituitary abnormalities and a predisposition to MPHD[180].

Transitional care

A period of transition in GHD is a shift between paediatric care to the adult treatment regimen occurring from the mid-teens to late teens, up until the mid-twenties. Establishing an appropriate consultation before the end of the paediatric age is essential as the interval between paediatric care and adult care is often associated with non-attendance and consequent loss to follow up by health-care professionals[181].

Persistent or transient GHD. Patients should be categorized according to their risk of persistent GHD. The current guidelines for GH testing during transition all agree on the need for retesting patients with IGHD after stopping rhGH for at least 1 month[16,182]. However, patients with idiopathic IGHD and an IGF1 of ≥0 SDS probably do not have persistent GHD, and hence transition therapy might not need to be considered[183]. Various causes for normal GH responses upon retesting in IGHD can be hypothesized. Some patients might have a partial GHD, which is sufficient to cause short stature during childhood but does not meet the stricter criteria for diagnosing GHD in adulthood[182]. In others, GHD might have been transient. Additionally, the low reproducibility of provocative tests might have a role. A lack of priming with sex steroids before testing in peripubertal children might also contribute to a discrepancy in testing between childhood and adulthood[183], as can changes in BMI over time. Finally, patients with brain trauma might have transient GHD[184].

Increased likelihood of persistence is seen in patients with an early age at diagnosis, anatomical, organic or genetic causes of GHD, and MPHD. Repeating a GH stimulation test is not necessary in patients with any of the following factors: MPHD (three or more hormonal deficiencies), low serum concentrations of IGF1 (less than −2.0 SDS), documented genetic defects affecting pituitary function and/or hypothalamic–pituitary structural brain defects. In these patients, rhGH therapy can be continued without interruption, although the dose needs to be reduced to adult age dosing, which is lower than weight-based dosing in children[16,182]. By contrast, in patients with a history of brain radiation, GHD might occur up to 10 years after exposure and therefore these individuals might have GHD despite normal growth[185].

GH stimulation testing during transition. The guideline for provocative testing varies according to society and government-sponsored guidelines. The insulin tolerance test remains the gold standard. An appropriate hypoglycaemic stimulus is considered when glucose drops below 2.78 mmol/l (50 mg/dl) and is associated with symptoms[16,182]. A peak GH response of <5 μg/l has approximately 95% sensitivity and specificity to detect GHD[186]. This method needs close monitoring as severe neuroglycopenic symptoms might develop and the test should be terminated if glycaemia falls below 35 mg/dl. This test is contraindicated in patients with a history of seizures, and cardiovascular or cerebrovascular disease. As a result of these safety concerns, this test has been used less frequently than it was originally. Depending on the availability, other tests can be used, such as GHRH in combination with arginine, glucagon, or the macimorelin stimulation test[182]. For glucagon, a GH cut-off of <3 μg/l is recommended in those with a normal BMI (18.5–24.9 kg/m^2) and decreases to <1 μg/l in those with a BMI of >30 kg/m^2 and low pretest probability. The cut-off to be used for BMI between 25 and 30 kg/m^2 is controversial. For the GHRH and arginine test, the cut-off peak values vary widely between studies from 5.6 μg/l to 20.3 μg/l, as BMI-adjusted clear cut-offs have not been established yet for adolescents and young adults[187]. For the macimorelin stimulation test, which was approved in 2019, a GH cut-off of 2.8 μg/l was recommended by the FDA[182]. A 2021 report suggests that this test is not influenced by BMI and recommends 5.1 μg/l as the best cut-off[188].

Treatment with rhGH during transition. Throughout transition, rhGH treatment enables patients to reach an appropriate level of somatic development, induces increases in lean mass, normalizes metabolism and improves quality of life[181,182,186]. Stopping treatment, although not recommended, should be at least accompanied by monitoring GH-dependent end points. GHD in adults results in decreased quality of life, increased risk of bone fracture, increased concentrations of LDL cholesterol and decreased concentrations of HDL cholesterol[16,181,182,186]. Although some question the efficacy of rhGH for protecting against osteoporosis, most believe that replacement therapy protects against its development[189,190]. Similarly, GH is needed for maintaining healthy body composition, as cessation of treatment leads to an increase in visceral

adipose tissue mass[191,192]. Changes in body composition in adolescents with severe GHD were demonstrated after only 6 months off therapy, with increased relative and absolute adipose tissue mass, and loss of lean body mass[191]. Standard lipid profiles improve with rhGH, with decreases in total and LDL cholesterol[182,193,194].

In terms of glucose metabolism, the association between type 2 diabetes mellitus (T2DM) and rhGH treatment remains controversial. Untreated patients might be more predisposed to T2DM due to increased visceral adipose tissue mass; however, GH per se is a counter-regulatory hormone as it antagonizes the hepatic and peripheral effects of insulin on glucose metabolism via mechanisms that involve an increase in free fatty acids[182,195–197]. Concerns around the development of T2DM appeared in the Kabi International Metabolic Survey database (now known as the Pfizer International Metabolic Database), which showed an increased prevalence of T2DM, but were invalidated in the Hypopituitary Control and Complications Study when risk factors such as age, sex and BMI were accounted for[197,198]. The current evidence does not provide enough data to show a causal relationship between rhGH and T2DM[182,184,185,196–199]. If T2DM is suspected throughout treatment, addition and/or adjustment of antidiabetic medications and reduction in rhGH dosing is suggested, although withholding rhGH treatment and focusing on achieving optimal glycaemic control is also a reasonable strategy before resuming rhGH therapy[182].

Although no major cardiac function abnormality has been observed after GH discontinuation, an improvement in markers of endothelial dysfunction and positive effects on left ventricular mass, interventricular septum, diastolic function and stroke volume index have been reported with rhGH therapy[182,200,201]. However, a 2021 report from a Swedish nationwide cohort of 3,409 adults with IGHD treated with rhGH since childhood showed an increase in the adjusted hazard ratio for all cardiovascular events when compared with individuals matched for age and sex[202]. The reason behind this increase could stem from GH treatment, persistent but untreated GHD in adulthood, other conditions being treated, other potential confounders not captured, or by a combination of the above[203]. Importantly, the consequences of GHD on life expectancy have been questioned by observations in a specific population of patients with IGHD caused by a *GHRHR* mutation. Despite untreated lifetime GHD, these individuals did not have evidence of premature cardiac or cerebrovascular atherosclerosis even in old age while maintaining normal life expectancy[204].

During transition, patients are treated with daily subcutaneous rhGH similarly to the paediatric population. However, as GH secretion varies during a lifetime, the dosing should follow the pattern determined by age and sex, along with any comorbidities and oestrogen status[16,182]. For patients younger than 30 years, most guidelines recommend initiating a dose of 400–500 μg per day, with a mildly increased dose during transition, that is, an increase in daily dosing by 100–200 μg per day every 1–2 months based on the individual's response[16,182]. Dosage of long-acting preparations will depend on the specific formulation. Importantly, women might need a higher dose than men, especially if receiving oral oestrogens, due to a first-pass effect in the liver, which renders the organ GH-resistant. For this reason, it is recommended that oestrogen replacement is administered via a transdermal patch in women on GH replacement. In terms of GHD aetiology, no difference between childhood-onset and adult-onset exists in rhGH dosing.

During transition, serum concentrations of IGF1 should be monitored every 4 to 6 weeks until the optimal maintenance dose of rhGH is achieved. A repeat follow-up IGF1 should be measured every 6 to 12 months. No consensus exists on the optimal target IGF1 concentration; however, in general, the goal is to maintain a concentration within age-specific and sex-specific normal ranges. As previously mentioned, serum concentrations of IGF1 in high quartiles have been associated with increased risk of certain malignancies in population studies; therefore, keeping IGF1 in the mid-range, rather than in the high–normal range, seems advisable. By using reduced GH dosages, such an approach could also limit the cost of therapy for health systems. In the future, the development of an index that would more closely correlate with long-term outcomes (such as HbA_{1c} in diabetes mellitus) would be ideal for adjusting GH dosing in young adults, in whom growth cannot be used as the ultimate measure.

Conclusions

In conclusion, great advances have been made in the past decades in refining the diagnosis and determining the causes of childhood GHD, while optimizing its treatment. The contribution of neuroimaging has led to the identification of specific pituitary and brain abnormalities. This advance has enabled the characterization of patients to be screened for additional pituitary deficiencies, those who might need GH replacement in adult life and those who need molecular studies and genetic counselling. Along with new technologies, such as next-generation and possibly whole-genome sequencing, improvement in the molecular diagnostic process progresses at an impressive pace. Various questions remain that need to be answered, including the variable penetrance of genetic mutations, the considerable phenotypic variability, the role of environmental factors and the interaction between candidate genes, which suggests a notable role of digenicity or oligogenicity. The development of therapeutic long-acting GH preparations holds the promise of being an effective treatment that would overcome the problems of poor adherence associated with the burden of daily injections. Long-acting GH preparations, thus, might have different effects on efficacy, metabolism and safety; with the factor of safety still a matter of investigation, particularly in patients treated with high doses. The problems associated with the poor reproducibility of GH stimulation tests are as yet partially unsolved and remain a major challenge in diagnosing GHD, particularly in children with IGHD; however, our knowledge of GH secretory dynamics has considerably expanded in the past few decades. The relative importance of MRI and a molecular diagnosis in these patients might be particularly worth pursuing.

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The GHRH signal disruption syndrome in a cohort followed for 26 years has been a valuable model to study the role of GH in body size and function.

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C.H., H.-W.G., A. I. and G.P. researched data for the article. R.S., M.D. and S.L. contributed substantially to discussion of the content. C.H., H.-W.G., A.I., G.P. and M.M. wrote the article. R.S., M.D., S.L. and M.L. reviewed and/or edited the manuscript before submission.

Competing interests
R.S. has served on advisory boards for Novo Nordisk and Ipsen. M.D. has served on advisory boards for Novo Nordisk, Pfizer and Ipsen and has received consulting/lecture fees from Sandoz, Pfizer and Novo Nordisk. M.M. has served on advisory boards for Ascendis, Biomarin, Merck, Novo Nordisk, Pfizer and Merk and has received lecture fees at several meetings. S.L. has received lecture fees and served on advisory board for Merck Serono, Ipsen and Sandoz. The other authors declare no competing interests.

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