Growth hormone — past, present and future

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Abstract | Growth hormone (GH) research and its clinical application for the treatment of growth disorders span more than a century. During the first half of the 20th century, clinical observations and anatomical and biochemical studies formed the basis of the understanding of the structure of GH and its various metabolic effects in animals. The following period (1958–1985), during which pituitary-derived human GH was used, generated a wealth of information on the regulation and physiological role of GH — in conjunction with insulin-like growth factors (IGFs) — and its use in children with GH deficiency (GHD). The following era (1985 to present) of molecular genetics, recombinant technology and the generation of genetically modified biological systems has expanded our understanding of the regulation and role of the GH–IGF axis. Today, recombinant human GH is used for the treatment of GHD and various conditions of non-GHD short stature and catabolic states; however, safety concerns still accompany this therapeutic approach. In the future, new therapeutics based on various components of the GH–IGF axis might be developed to further improve the treatment of such disorders. In this Review, we describe the history of GH research and clinical use with a particular focus on disorders in childhood.

The great complexity of the growth hormone (GH)–insulin-like growth factor (IGF) axis is mirrored by the wide range of growth disorders corresponding to different pathophysiological circumstances. Indeed, a vast number of scientists and clinicians have advanced the field of GH research for more than a century, reflecting its clinical importance.

In addition to GH deficiency (GHD) or GH excess, a multitude of other growth disorders within the GH–IGF axis have emerged. The diagnostic procedures that have been developed alongside have influenced the clinical management of patients thought to have pituitary pathology, particularly those with GHD. The introduction of recombinant human GH (rhGH) has ended the era of quantitative limitations and risks that characterized the previous use of pituitary-derived human GH (pit-hGH)[1]. Additionally, rhGH has now been shown to be effective in many non-GHD growth disorders[2]. In these disorders, as well as in catabolic states, rhGH represents a surrogate therapy until more pathophysiologically appropriate approaches are available.

To date, treatment optimization of daily injected rhGH therapy has been attempted by adjusting the rhGH dose to the expected sensitivity on the basis of clinical predictors or the effect on serum IGF1 levels during treatment. Such an approach appears to decrease the variability of the growth response to rhGH[3]. In parallel, various rhGH preparations and GH analogues with an extended action have been developed in the past 10 years aiming at a similar efficacy but a lower patient burden. In addition, some structural modifications of the GH molecule have been shown to antagonize its action[4]. Beyond the treatment of acromegaly, antagonists of the GH–IGF axis might have potential for the prevention or treatment of malignancies in the future. After decades of clinical and basic research, it is remarkable that new insights into the GH–IGF system are continuously emerging in parallel with new technologies, suggesting that novel diagnoses and therapies will be related to this system in the future.

In this Review, we describe the history of GH research and clinical use, from its discovery and purification to the recognition of its various effects to the first therapeutic use of pit-hGH. The research developments and discoveries are presented in relation to their clinical context. Furthermore, the emerging physiology and pathophysiology of the GH–IGF axis are discussed, with particular focus given to the related disorders in childhood. In addition, the use of rhGH in various growth disorders, the potential risks of GH therapy and the emerging new therapeutic tools are highlighted. The role of GH and its related disorders in adults is only briefly touched upon.

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Key points

- The growth hormone (GH)–insulin-like growth factor (IGF) axis consists of central neuro-anatomical, regulatory and genetic systems, and the peripheral intracellular GH signalling pathway
- The introduction of recombinant human GH (rhGH) in 1985 ended the phase of pituitary-derived human growth hormone (hGH) and its associated limitations and risks, opening the possibility of widespread clinical use
- GH deficiency (GHD) is a syndrome with many different causes and is associated with alterations in growth, body composition and metabolism
- In some non-GHD short stature disorders, rhGH has been proved effective and is used as a surrogate for the currently unknown, pathophysiologically appropriate treatment
- Risks of hGH therapy might relate to its direct effects on growth, its anti-insulin action and its cell-proliferating activity; however, the safety profile of rhGH in children and adults is good
- Current innovative treatment approaches relate to personalizing conventional rhGH, developing long-acting GH preparations, the prospect of gene therapy, GH–GH receptor antagonists and, potentially, new indications

Discovery, purification and synthesis

Extremes in stature were initially not seen from the medical perspective but rather as an anomaly and curiosity depicted in the arts or even exhibited in circuses or 'freak shows' during the 19th century. In particular, two Americans, the 'dwarf' Charles S. Stratton (alias General Tom Thumb; adult height 101 cm) and the 'giant' Lewis Wilkins (adult height 245 cm), gained international recognition and fame, not least because picture postcards of them were distributed[5].

Research into the function of the pituitary, and GH in particular, started with clinical observations and anatomical descriptions of people with gigantism and adults with acromegalic features[6]. In 1884, the Swiss general physician Fritsche reported in great detail the history of a 44-year-old man developing the characteristic features of acromegaly — a term later coined by Pierre Marie in 1886 (REF. 7) — and an enlarged pituitary, which was observed post-mortem[8]. Minkowski, who is famous for the discovery of the pancreatic origin of diabetes mellitus, proposed the connection between the pituitary and acromegaly before eosinophilic tumours of the anterior pituitary emerged as the anatomical basis of gigantism and acromegaly[9]. The clinical appearance of short stature with obesity caused by a tumour of the hypothalamo–pituitary region (dystrophia adiposogenitalis), which was recognized independently by Babinski and Fröhlich, suggested that the pituitary gland has other effects apart from promoting growth[10,11]. Cushing, based on his experience as an eminent neurosurgeon, conceived the existence of a pituitary 'growth hormone' (REF. 12) and actively promoted research in search of specific pituitary hormones.

The recognition of the effects of pituitary GH and its purification and chemical synthesis in various species is closely related to the work of Evans and his eminent research associates at the University of California, San Francisco[13]. In 1921, Evans and Long[14] demonstrated the growth-promoting effect of bovine pituitary GH extracts in rats, even beyond normal size. In the search for other actions of anterior pituitary extracts, a variety of metabolic effects were elucidated. The diabetogenic effect of anterior pituitary extracts in a dog model was observed by Houssay and Biassotti in 1930 (REF. 15) and led to extensive research into glucose metabolism in humans. In addition, the effects of GH on the metabolism of glucose, proteins, minerals and lipids were studied[16]. It was observed that a lack of GH leads to an increase in insulin sensitivity with the risk of hypoglycaemia. In addition to the lipolytic effect, an increase in nitrogen storage indicating protein accretion and the retention of potassium, phosphorus, calcium and sodium was observed with GH, proposing potential clinical applications of the hormone.

Li, the leading chemist within Evans' group, isolated bovine and human pituitary GH to purity, described its primary structure — a protein of 191 amino acids with two sulfide bonds — and finally achieved the chemical synthesis of hGH[17,18]. During the 1940s and 1950s, GH preparations from different species were purified and tested in animals and humans. Guided by the experience gained with insulin, researchers expected a ubiquitous effect of their preparations. However, although bovine GH was shown to be effective in rats, it had no effect in humans[17]. It was Ernest Knobil who eventually recognized the species specificity of GH in primates[19]. The incompatibility of the His171 residue in nonprimate GH with the Arg43 residue in the human GH receptor (GHR) was later found to be the main reason for this unique phenomenon[20]. A timeline of the main historical events in GH research until 1957 is shown in FIG. 1.

After GH purification had been optimized, a new era started following the first successful use of pit-hGH in 1958 by Raben and Beck[21]. Raben reported that 2 mg of a pit-hGH extract given three times per week to a 17-year-old pituitary dwarf (the terminology used at the time) resulted in a growth rate of 2.6 inches per year, compared with 0.5 inches per year before treatment[22]. Beck reported in a 13-year-old male with well-documented hypopituitarism due to craniopharyngioma that human and monkey GH resulted in a substantial increase in nitrogen storage, a retention of potassium, phosphorus and calcium and a gain in body weight during two treatment periods.

After pit-hGH was proved effective, the demand and competition for human pituitaries increased dramatically. In 1960, Blizzard and other experts founded the National Pituitary Agency (NPA) in the USA for the collection of pituitaries and the purification and distribution of GH[23]. An estimated 6 million international units (IU) of pit-hGH have been made available for clinical studies over the years[24]. Similar national systems were established in other countries, and purifying and commercializing pit-hGH were organized by pharmaceutical companies. From 1963 to 1985, commercial pit-hGH preparations isolated from frozen glands using appropriate isolation techniques were proved to be free of contamination by other pituitary hormones[25]. However, the limited availability of pit-hGH placed a restriction on the pace of advancement in knowledge in this field. The main historical highlights in GH research from 1958 until the present day are shown in FIG. 2.

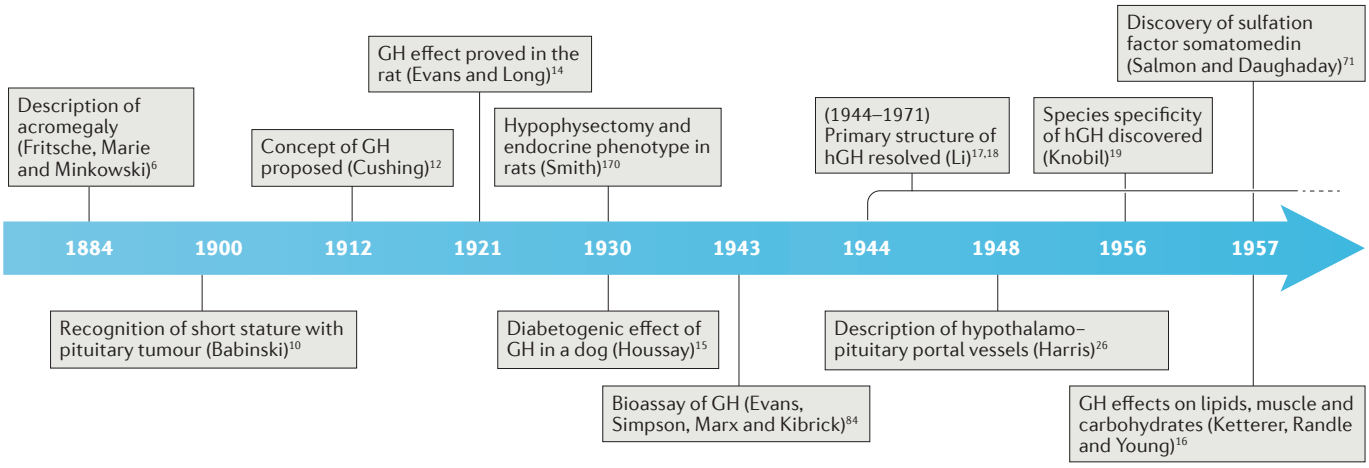

Fig. 1 | **Timeline of important discoveries in GH–IGF axis research until 1957.** GH, growth hormone; hGH, human GH.

The GH–IGF axis

The cascade from GH synthesis, including its anatomical and molecular basis, to its cellular and partly IGF-mediated actions is briefly termed the 'GH–IGF axis'; however, in many aspects, it functions rather as a network. For succinctness in this section, the discussion of the various physiological and pathophysiological aspects of the GH–IGF axis at different levels has been intertwined. The focus of the discussion on the GH–IGF axis herein is directed towards the regulation of hGH secretion at the genetic, cellular and neuroendocrine level, GH signal transduction and the functions of IGFs and their binding proteins. For an overview of the GH–IGF axis and GH signalling, see FIG. 3.

Ontogeny of the pituitary

The early anatomical studies by Harris in the 1940s laid the groundwork for the insight that production of pituitary hormones (for example, GH) requires the anatomical and functional integrity of the hypothalamo–pituitary portal vessels and neural structures[26]. The ontogenetic development of the pituitary, with the posterior region and the infundibulum (stalk) being derived from the neuroectoderm and the anterior region from the oral ectoderm, involves a number of factors that are under complex genetic control[27]. Pituitary malformations associated with other brain malformations, such as Rathke cleft cyst, septo-optic dysplasia (SOD) or holoprosencephaly, are causes of congenital hypopituitarism[28]. Defects in *HESX1* (which encodes homeobox expressed in ES cells 1) were shown to cause SOD in humans and mice[29], and defects in *LHX3* (which encodes the LIM homeobox protein 3) lead to pituitary aplasia in mice and to hypopituitarism combined with a stiff neck in humans[30]. The pituitary stalk interruption syndrome, characterized by an interrupted pituitary stalk, ectopic posterior pituitary and hypoplasia and/or aplasia of the anterior pituitary, is an example of a developmental anomaly that exclusively affects the pituitary region and can be caused by defects in *HESX1* or *LHX4* (REF. 31).

The development of cells of the anterior pituitary from progenitors to cells that are specialized in the production of hormones — such as somatotrophs for GH production — occurs ontogenetically in a specific sequence. This process of enormous complexity is governed by transcription factors under genetic control[27]. Mutations in genes encoding such transcription factors lead to different patterns of combined pituitary hormone deficits. Soon after the discovery of mutations in *POU1F1* (which encodes pituitary-specific positive transcription factor 1)[32] in patients with GH, TSH and prolactin deficiency, mutations were found in the gene encoding the pituitary-specific transcription factor homeobox protein prophet of Pit 1 (PROP1), which has a role in the development of almost all anterior pituitary hormone-producing cells[33]. *PROP1* mutations represent a relatively frequent cause of hypopituitarism[34]. In addition, immunoglobulin superfamily member 1 (IGSF1) deficiency is primarily associated with central hypothyroidism and prolactin deficiency, but transient partial GHD has been observed in 14% of children with IGSF1 deficiency[35]. All reported genetic causes of GHD are listed in Supplementary Table S1.

The hGH network

After the primary structure of hGH had been resolved[18], the three-dimensional structure revealed the existence of four α-helices, nonhelical chains, two disulfide bridges and a hydrophobic core[36]. GH has two GHR binding sites located on opposite sides of a bundle of α-helices that interact with GHR molecules[37].

The gene cluster containing the hGH genes (*GH1* and *GH2*) and the human chorionic somatomammotropin genes (*CSH1, CSH2* and *CSP1*) resides on the long arm of chromosome 17q23.3 (REF. 38). *GH1* encodes the 24 kDa long pre-hGH transcript, which yields the predominant hGH variant of 22 kDa (191 amino acids) after processing of the primary transcript or a 20 kDa variant (lacking amino acids 32–46) by alternative splicing (in 10% of transcripts)[38]. The *GH2* gene product is a 22 kDa hGH variant (hGH-V) that is expressed only in the

Hypothalamo–pituitary portal vessels
A system of blood vessels connecting the hypothalamus with the anterior pituitary.

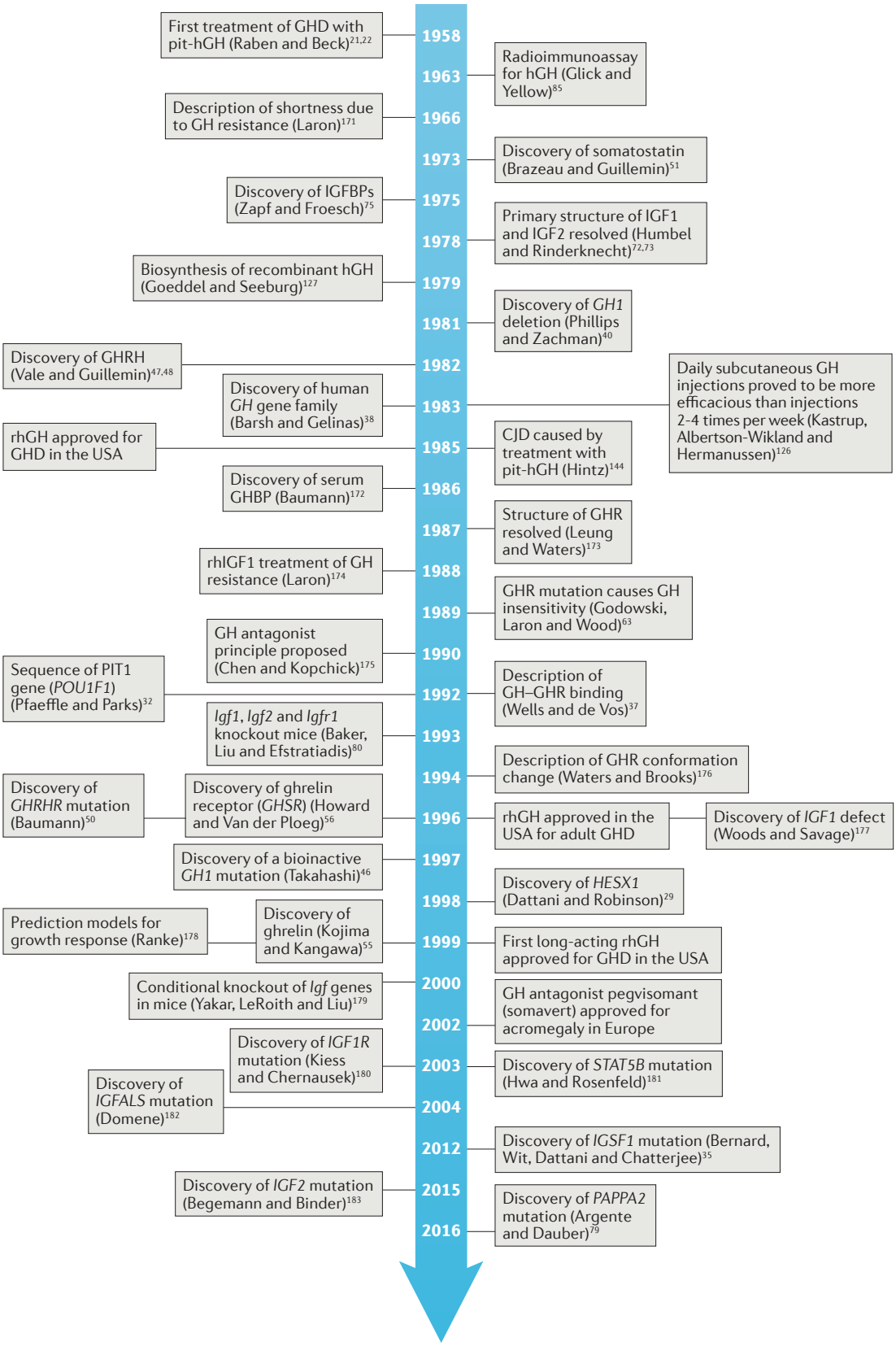

Fig. 2 | **Timeline of important discoveries in GH–IGF axis research from 1958 to present.** CJD, Creutzfeldt–Jakob disease; GH, growth hormone; GHBP, GH binding protein; GHD, GH deficiency; GHR, GH receptor; GHRH, GH-releasing hormone; hGH, human GH; IGF1, insulin-like growth factor 1; IGF2, insulin-like growth factor 2; IFGBP, IGF binding protein; pit-hGH, pituitary hGH; rhGH, recombinant human GH; rhIGF1, recombinant human IFG1.

Isolated GHD
(IGHD). Defined by the selective lack of pituitary growth hormone secretion in contrast to normal secretion of other pituitary hormones.

syncytiotrophoblast of the placenta and released into the maternal circulation and has a role in feto–maternal development[39].

In addition to these genetically expressed forms of hGH, there is great variety in post-translational modification of hGH in blood, including N(α)-acylated, deamidated and glycosylated monomeric GH forms, as well as both non-covalent and disulfide-linked oligomers. Their functional role is largely unclear[39].

GH1 mutations cause inherited forms of isolated GHD (IGHD) with eutopic pituitaries and are usually classified according to their mode of inheritance (see Supplementary Table S1). Individuals with IGHD type IA, an autosomal recessive disorder defined by the absence of GH levels due to a 7.5 kb deletion including the complete *GH1* gene, develop growth-attenuating anti-GH antibodies when treated with hGH[40,41]. A less severe autosomal recessive condition, IGHD type IB, is the result of splice site, frameshift or nonsense mutations in *GH1* or mutations in *GHRHR* (which encodes the GHRH receptor (GHRHR))[42]. The autosomal dominant IGHD type II is caused by heterozygous splice site, missense or splice enhancer mutations or intronic deletions in *GH1*, which result in skipping of the third exon and a consequent 17.5 kDa GH variant that exerts a dominant-negative effect on GH secretion[43]. The X-linked IGHD

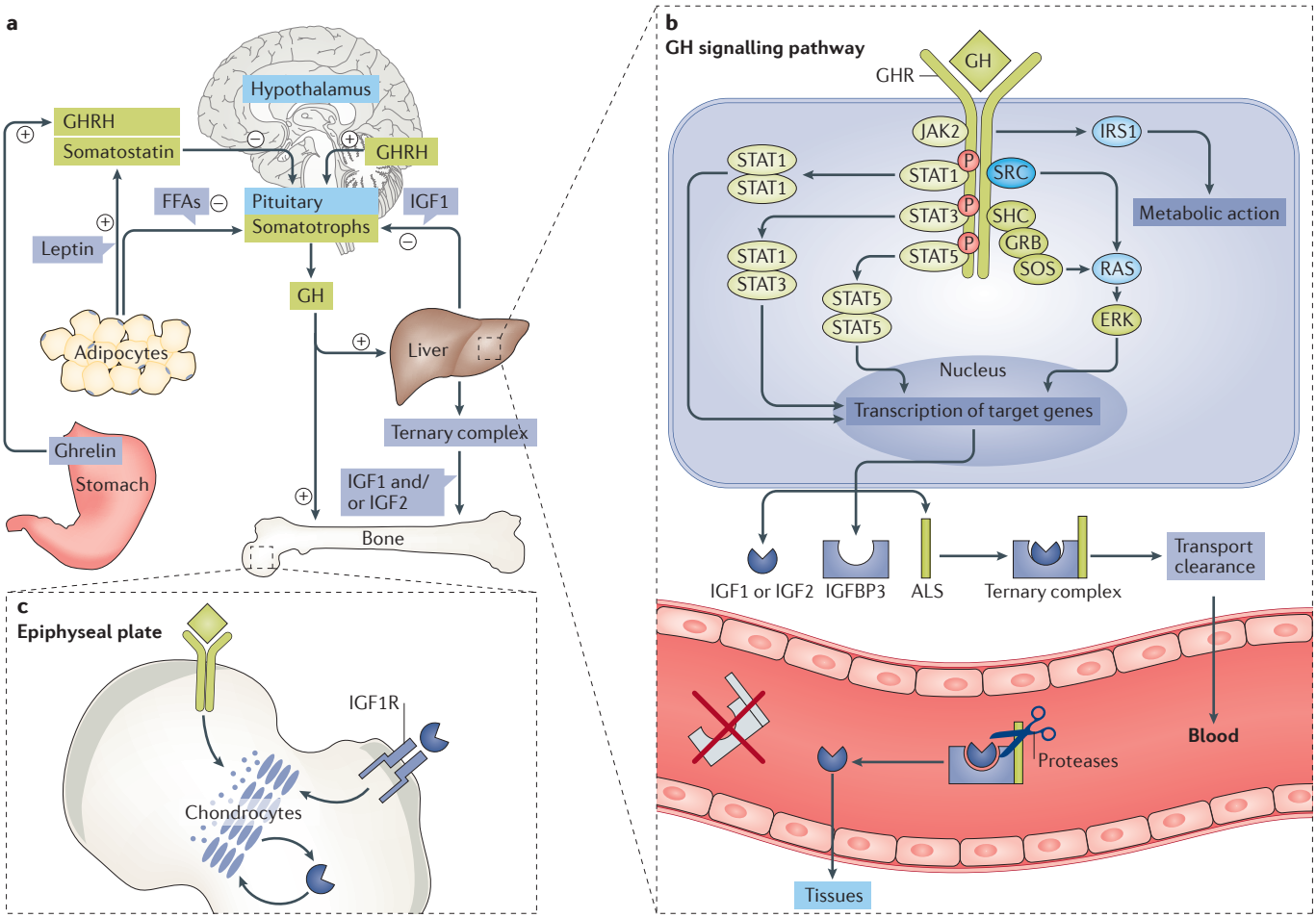

Fig. 3 | **The GH–IGF axis and GH signalling pathway. a** | Growth hormone (GH) is secreted in a pulsatile fashion from the pituitary somatotrophs under the positive (+) and negative (–) influence of the hypothalamic hormones GH-releasing hormone (GHRH) and somatostatin, respectively. GHRH is activated by ghrelin, whereas adipocytes inhibit GH secretion by leptin-mediated somatostatin secretion and by a direct effect of free fatty acids (FFAs) on the pituitary somatotrophs. GH acts on multiple cell types, tissues and organs, but for growth, its main targets are the liver and the epiphyseal plates in long bones and spine. **b** | In all cells that express the GH receptor (GHR), binding of GH leads to increased expression of a number of genes via a complex signalling pathway. With respect to growth, the main pathway in the liver is activation of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 5B (STAT5B), leading to increased expression of genes encoding insulin-like growth factor 1 (IGF1) and IGF2, IGF-binding protein 3 (IGFBP3) and IGFBP5 and acid labile subunit (ALS), which enter the circulation in the form of the so-called ternary complex. IGF1 and IGF2 are liberated from this ternary complex by proteases, notably pappalysin 2 (PAPPA2), which enables them to pass through the capillary epithelium and enter the interstitium. Circulating IGF1 serves as a negative factor for GH secretion in the pituitary gland and has a general anabolic function on almost all cell types. **c** | The chondrocytes in the epiphyseal plate are stimulated not only by IGF1 and IGF2 but also directly by GH, and proliferative and hypertrophic chondrocytes also secrete IGFs (autocrine regulation). ERK, extracellular-signal-related kinase; GRB, growth factor receptor-bound protein; IRS1, insulin-like growth receptor 1; SHC, SHC-transforming protein; SOS, son of sevenless homologue; SRC, proto-oncogene tyrosine-protein kinase Src.

type III (X-linked hypogammaglobulinaemia and isolated GHD (XLH-GHD)) is characterized by GHD plus agammaglobulinaemia[44].

The first suggestion that naturally circulating hGH could be altered such that it was measurable by immunoassay but had no, or minimal, bioactivity was made by Kowarski and colleagues in 1978 (REF. 45). Much later, point mutations in *GH1* were reported to cause bioinactive GH variants in short children, who responded successfully to rhGH treatment[46].

In the next paragraphs, the three main regulators of GH secretion and mechanisms of GH secretion are discussed.

GH-releasing hormone. In 1982, peptides that stimulated GH production and release from pituitary somatotrophs were isolated from pancreatic tumours causing acromegaly[47,48]. The peptides — GRF(1–40)NH_2, GRF(1–37)NH_2 and (the major component) GRF(1–44)NH_2 — were first termed 'GH-releasing factor' (GRF) and later GH-releasing hormone (GHRH) and exerted their effects on somatotroph proliferation and GH secretion via a G protein-coupled receptor, GHRHR, in the presence of the pituitary-specific transcription factor POU1F1 (REF. 49) (FIG. 3a). A point mutation in *GHRHR* was found to be the cause of the GH-deficient dwarf (lit/lit) mouse, and analogous gene defects were found to cause severe GHD in humans[50].

Somatostatin. In 1973, a GH-inhibiting peptide termed 'somatropin-release inhibiting factor' (SRIF; now known as somatostatin) was isolated from thousands of sheep hypothalami[51]. The 14 or 28 amino acid peptides are produced in neurons of the ventromedial nucleus of the hypothalamus and inhibit GH secretion via the G protein-coupled somatostatin receptor (SSTR) on somatotrophs (FIG. 3a). Five SSTR subtypes (SSTR1–SSTR5) are known, of which SSTR2 is the principal receptor on somatotrophs[52]. Somatostatin secretion inhibits TSH and prolactin secretion by the pituitary[53]. Somatostatin also exerts inhibitory effects on the expression of various other hormones in the gut and other parts of the body[54]. IGF1 and the somatostatin antagonist GHRH augment hypothalamic somatostatin release, and somatostatin inhibits the pituitary GH secretion provoked by GHRH[53].

Ghrelin. In 1999, the GH secretagogue (GHS) ghrelin, a 28 amino acid peptide with a unique *N*-octanoylation modification at Ser3, was discovered[55]. Ghrelin affects GH secretion and energy homeostasis and is mainly produced from its precursors in cells of the gut but also in the ventromedial and arcuate nucleus of the hypothalamus. Ghrelin is acetylated by ghrelin *O*-acyltransferase (GOAT) to the major component C-ghrelin, which transmits its biological signals (for example, modulation of GHRHR-related GH release) via a GHS receptor (GHSR) isoform, GHSR1a[56] (FIG. 3a). Ghrelin levels in the blood are high in the fasting state and low after feeding. Ghrelin and leptin are considered to form an essential link between the gut, energy homeostasis and neuroendocrine regulation of GH and gonadotropins[57].

Mechanisms of GH secretion. GH is secreted in a pulsatile mode with peaks (higher at night than during the day and mediated by a reduction in tonic hypothalamic somatostatin secretion) and troughs (FIG. 3a), as assessed by frequent sampling (every 5–20 minutes)[58]. With the deconvolution technique developed by Veldhuis[58], many factors affecting the quality and quantity of GH secretion (for example, age, sex hormones and nutritional status) have been investigated in children, adolescents and adults. GHRH, somatostatin and ghrelin and their successive cascades of action are the most important players within the network of factors regulating GH secretion[59]. Importantly, there might be different roles of GH troughs (body composition) and peaks (growth) in humans[60].

GH signal transduction

The discussion of GH signal transduction considers the GHR, the GH binding protein (GHBP), the GH–GHR interaction and the subsequent intracellular signalling cascades.

GHR and GHBP. GH binding to its cell surface receptor, GHR, initiates an intracellular signalling cascade. The GHR is a member of the class 1 haematopoietic cytokine receptor family and consists of an extracellular region for GH binding (246 amino acids in length), a helical transmembrane region (24 amino acids in length) and an intracellular domain (350 amino acids in length), the latter of which mediates the intracellular transmission of GH binding signals[61]. The *GHR* gene is located on chromosome 5p13 and consists of ten exons. A common GHR polymorphism is deletion of exon 3 (GHRd3), which is part of the gene region (exons 1–7) encoding the extracellular domain, resulting in a GHRd3 isoform that is probably more sensitive to GH than wild-type GHR[62].

Initially, it was thought that dimerization of the GHR occurred after a GH molecule was trapped by one GHR[37]. However, it was later shown that GH binds to a GHR dimer, which then changes its conformation and transduces the signal[61] (for an animation, see Related links). *GHR* mutations in the extracellular, transmembrane and intracellular regions cause the autosomal recessive and GH-insensitive Laron syndrome[63].

In humans, the extracellular domain of the GHR can be cleaved by the metalloproteinase TNF-converting enzyme (TACE; also known as ADAM17) and circulates in blood as GHBP[64]. The concentration of GHBP in blood is thought to reflect the GHR expression status. GHBP binds ~50% of the circulating GH and prolongs the half-life of GH, therefore serving as its buffer; however, its physiological role is incompletely understood[64]. Depending on the position of the *GHR* defect, GHBP might be absent (~75% of cases) or present in normal or even increased concentrations in patients with Laron syndrome[65].

GH intracellular signal transduction. Following GH–GHR binding, several intracellular signalling pathways are activated, such as the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, among others (FIG. 3b). Activation of tyrosine kinases of the JAK family (predominantly JAK2)[66] leads to activation

Agammaglobulinaemia
A term for deficiencies of immunoglobulins that electrophoretically migrate into the γ-fraction.

Laron syndrome
A growth disorder due to an insensitivity to growth hormone caused by a mutation in the growth hormone receptor.

of STAT proteins (particularly STAT5b), which induces their dimerization and nuclear translocation, where they promote the transcription of various genes, such as those encoding IGF1, IGF-binding protein 3 (IGFBP3), acid labile subunit (ALS) and IGF2 (REF. 67) (FIG. 3b). Other pathways activated by GH include the mitogen-activated protein kinase (MAPK)–extracellular-signal-regulated kinase (ERK) pathway[68]. The internalization of the GH–GHR complex via the ubiquitin-proteasome system[69] is part of the degradation of GH, but the complex might even result in intracrine hormone actions[69,70].

The IGF–IGFBP system

In 1957, Salmon and Daughaday[71] found that in vitro sulfate incorporation into cartilage by serum was not a direct but rather an indirect effect of GH. This so-called sulfation factor was later termed 'somatomedin', an entity grossly defined as being GH-dependent, insulin-like and mitogenic. Somatomedins were found to be identical to known insulin-like factors (IGF1 and IGF2), which were termed according to their similarity to proinsulin[72,73] and became the official nomenclature. The functions of IGFs are to increase cell proliferation, promote tissue-specific cell functions, exert metabolic effects similar to insulin and evoke anti-apoptotic actions[74].

Subsequent research revealed that the IGF system also consisted of two receptor tyrosine kinases, IGFR1 (which is similar to the insulin receptor) and IGFR2, and six structurally similar IGFBPs (IGFBP1–IGFBP6)[75] that have an affinity for IGFs of approximately one order of magnitude higher than that of IGFs for IGFRs[76] (FIG. 3). The IGFBPs bind to IGFs, preventing IGFs from being degraded and facilitating their transport through body compartments. IGFBPs can also bind to cellular structures, tissue matrix proteins and peptides, such as proteoglycans and integrins. This binding reduces the affinity to bound IGFs, thus rendering IGFs available for action. Independently of IGFs, IGFBPs were found to stimulate cell proliferation and might have additional effects[74,77].

Key components required for postnatal growth are IGF1, IGFBP3 and the ALS, which are all GH-dependent and form a large (150 kDa) ternary complex (FIG. 3b). IGFBP3 can be post-translationally modified. For example, the glycosylation or phosphorylation of IGFBP3 increases the binding of IGFs, whereas proteolysis, dephosphorylation or deglycosylation decreases IGF binding, thereby regulating IGF availability to its targets[78]. IGFBP3 is specifically cleaved by metalloproteinases (for example, pappalysin 2 (PAPPA2))[79], which liberates IGF1 from the ternary complex (free IGF1) and makes it available for the IGF1Rs (FIG. 3b). All elements of this complex cascade can modify IGF functionality and are therefore potential targets for novel pharmacotherapy.

In the 1980s, whether GH or IGFs function as the prime promoter of linear growth was discussed, whereas the endocrine versus paracrine and/or autocrine role of IGFs was debated in the 1990s. Later animal experiments using liver-specific *Igf1* deficient (LID) mice and other genetically manipulated mouse models confirmed that both GH and IGFs have a substantial and partly endocrine-mediated function in growth regulation[80,81].

Defining and diagnosing GHD

The definition and diagnosis of GHD require the discussion of its clinical context at the various phases of life (childhood, transition from adolescence to adult life and adulthood) and the biochemical tools and methods used to verify an impairment in GH secretion.

GHD in children

GHD in children can be congenital or acquired and either isolated or combined with other pituitary hormone defects. There are many well-defined causes of GHD in children, but the cause is often unknown (idiopathic GHD). The definitions of the various types of GHD and other causes of short stature are given in the International Classification of Pediatric Endocrine Diagnoses (ICPED). If untreated, childhood-onset GHD leads to permanent short stature. In children, the diagnosis of GHD rests on a detailed medical history, clinical features, growth (auxological) analysis, biochemical tests of components of the GH–IGF axis and radiological assessment of skeletal maturation and of pituitary anatomy using MRI[82].

As short stature (defined as height with a standard deviation score (SDS) <−2 for age and sex) is a frequent sign of GHD in children, a diagnosis requires all other potential causes of shortness (genetic, organic, hormonal, metabolic and/or psychogenic) to be excluded before a diagnosis of GHD should be considered[82]. In severe congenital GHD, height velocity is usually abnormally low from the first 6 months of life, which soon leads to low height SDS compared with population references and midparental height (MPH)[83]. Acquired (childhood-onset) GHD is mainly characterized by low height velocity[82]. In less severe cases, the likelihood of GHD is estimated based on a combination of the impairment of three main growth characteristics: height, height minus MPH and height velocity if compared with normal references for age and sex plus bone age delay[82]. Confirmation of GHD is established by biochemical investigations once auxological and radiological characteristics make the existence of GHD likely.

hGH measurement. Widening of the tibial epiphyses by GH treatment in adult hypophysectomized rats was initially used as a bioassay for GH activity, but this method was too insensitive for clinical use[84]. Soon after the publication of a labelling technique of hGH with ^{131}I, the first immunoassay for hGH was described in 1963 (REF. 85), and immunoassays are still widely used to date. Today, hGH levels in blood can be determined using various types of immunoassay, with a sensitivity that even permits measurements from filter paper used for neonatal screening[86], and more recently by mass spectroscopy[87,88].

One of the most relevant aspects for standardization when diagnosing GH secretory disorders[89] relates to changes in international reference preparations (IRPs). The first IRP for hGH (IRP 66/217) in 1969 was of pituitary origin with a designated specific activity of 2 IU/mg (REF. 90). A subsequent IRP (IRP 80/505) was based on purified pituitary GH, with a specific activity of 2.6 IU/mg, but the first and second biosynthetic GH IRPs (IRP 88/624 and 98/574) were purified 22 kDa recombinant

Midparental height
(MPH). The average height of the father and mother after converting them to standard deviation scores.

International reference preparations
(IRPs). International standard preparations (for example, for human growth hormone) are established by WHO experts (National Institute for Biological Standards and Control (NIBSC)).

hGH with an assigned potency of 3 IU/mg (WHO International Biological Reference Preparations)[90]. Thus, before 1982, 20 μIU hGH was equivalent to 10 ng but is only equivalent to 6.7 ng today[90]. In addition, different antibodies have been used in immunoassays. Initially, these antibodies were polyclonal and directed against all epitopes of pit-hGH, but after the 1990s, either polyclonal or monoclonal antibodies directed against different epitopes of monomeric 22 kDa rhGH were applied[90]. The latter approach might lead to the detection of lower hGH levels in human plasma.

Quantifying GH secretion. As a random measurement of plasma levels of GH is poorly reflective of GH secretion, multiple diagnostic procedures (GH stimulation tests) were developed to elicit GH secretion via various mechanisms at the hypothalamic or pituitary level, under the assumption that the maximum GH levels quantified in such tests could serve as a suitable diagnostic when compared with that of normal individuals.

The first recognized GH stimulus — still considered the gold standard, although many clinicians are reluctant to use it because of the potentially life-threatening complications — was insulin-induced hypoglycaemia using an insulin tolerance test (ITT)[91]. Of the 53 children with clinically evident GHD in one study, none exceeded the serum GH level of 5 μg/L (REF. 91), whereas the serum GH level exceeded 5 μg/L in 37 children without GHD in another study[92]. Thus, in the late 1960s, a serum GH concentration of 5 μg/L (then equivalent to 10 mU/L) was an accepted limit (cut-off) to distinguish GHD from non-GHD. In later years, it was common to accept the diagnosis if a maximum GH concentration of 7 mU/L (equivalent to 3.5 μg/L) was not surpassed in one test or if 7–15 mU/L (equivalent to 3.5–7.5 μg/L) was not surpassed in two standard tests[93,94]. After 1990, most physicians rather arbitrarily accepted a 10 μg/L cut-off. Only in recent years has evidence re-emerged that the cut-off should be near 7 μg/L for modern methods and references[94].

Many so-called standard test procedures and their combinations that induce GH secretion by applying various agents (for example, arginine, glucagon, propranolone and L-DOPA) have been published[94]. The varying mechanisms by which test agents act at the pituitary or hypothalamic level, their potential risks, the variability of response, the lack of reproducibility and the paucity of truly normative data have made this cut-off-driven diagnostic approach highly controversial. The quantification of spontaneous GH secretion by frequent sampling that was propagated in the 1980s is probably superior to stimulation tests, as this method reflects the physiological situation in an individual[95]. In healthy individuals, IGF1 and IGFBP3 levels have been found to be strongly and positively correlated with the amount of GH secreted[96]. The utility of serial sampling of GH for diagnosing GHD is, however, valued divergently because the results observed during GH testing or observed spontaneously might not be in agreement[97] and because of diagnostic traditions[95]. In addition, only a few clinicians are using serial sampling in clinical practice owing to practical and financial limitations of this diagnostic procedure. As physiological GH secretion tends to be relatively low before puberty, 'priming' with sex steroids shortly before performing GH stimulation tests has been advocated during the peripubertal age to avoid false-positive results[98].

IGF-related parameters. It has been proposed that GH and IGF1 are interrelated like other endocrine systems, with a centrally produced regulatory hormone (GH) and a peripherally produced and acting hormone (IGF1). Such a concept considers GHD as a special case of secondary IGF-deficiency (IGFD) caused by GHD, whereas primary IGFD includes GH insensitivity syndromes[99]. However, this model does not reflect the complex regulation and partly opposing physiological role of IGFs and IGFBPs. Although both IGF1 and IGFBP3 are GH-dependent and reflect spontaneous GH secretion[96], their levels in blood are influenced by other hormones (sex steroids, thyroid hormones and glucocorticoids), the immune system (cytokines) and, in particular, the nutritional status of an individual[96,100]. These aspects need to be considered when interpreting IGF1 and IGFBP3 levels.

However, it is of diagnostic advantage that the basal levels of IGF1 and IGFBP3 in blood are relatively stable. Some methodological standardization has been achieved for immunoassays of both peptides[87], and reference ranges for age and sex over the entire human lifespan have been published[101,102].

The value of serum IGF1 and IGFBP3 measurements for the primary diagnostic workup in childhood is well established[103] but is not undisputed. IGF1 (and, to a lesser extent, IGFBP3) levels are low in children with GHD but overlap partly with those of children who are short for other reasons[96]. Thus, truly normal levels (for example, IGF1 >−1.0 SDS) are unlikely in children with GHD, making this diagnostic parameter less specific than sensitive. If the diagnosis of GHD has been made, an MRI of the hypothalamus and/or pituitary region has become part of the standard workup of GH-secretory disorders to search for anatomical causes[104]. In cases with severe GHD, particularly if other members of the family have a similar phenotype, genetic evaluation is indicated[105].

Impaired sensitivity to GH, which can be tested by means of exposure to hGH (IGF generation test)[106], can be caused by defects in GH receptor binding (*GHR* mutations) or in the GH signal transduction cascade (*STAT5B* mutations) or by mutations in *IGFALS* (which encodes ALS) or *IGF1* (see Supplementary Table S1).

Neurosecretory dysfunction. Impaired spontaneous GH secretion associated with normal peak GH levels in stimulation tests was observed in children after cranial irradiation[107], which was assumed to be caused by hypothalamic damage with consequent GHRH deficiency. This so-called GH neurosecretory dysfunction (NSD) was suggested to exist in short children resembling the clinical phenotype of normal GHD but without known trauma to the hypothalamo–pituitary region

(idiopathic NSD), who are characterized biochemically by low spontaneous GH secretion and low IGF1 levels but normal GH levels in response to standard GH stimulation tests[108]. As very few clinicians assess spontaneous GH secretion, and because there is substantial overlap between spontaneous GH secretion of children with suspected GHD and healthy controls, at present, the term 'NSD' is rarely used as a diagnostic entity.

Demographics of childhood GHD. The causes of GHD are varied, but in most cases, the cause is unknown (idiopathic IGHD). Although before 1985 pit-hGH was almost exclusively administered to children with severe GHD, in whom the diagnosis was virtually certain, the loosened criteria (for example, higher cut-off for GH stimulation tests) in the past 30 years have not only led to an increased total number of patients receiving the diagnosis of GHD but also to an increasing percentage of less severe and even uncertain GHD. When patients with idiopathic GHD are retested in young adulthood, GHD is not confirmed in many cases[109]. Over a period from 1987 to 2012, the characteristics of patients defined as IGHD in Europe, the USA and Japan not only differed somewhat but also changed such that the frequency of males, the severity of the GH impairment and the height deficit decreased, indicating (unknown) socio-economic influences in the diagnostic process[110]. Reports on the prevalence of childhood-onset GHD in the order of 1 in 5,000 (with ~20% of cases being acquired) are only rough estimates[111].

GHD in transition

Many aspects of physical development (for example, body composition and reproductive maturation) continue beyond linear growth, which represents a phase before mature adult life termed 'transition'. Adolescents with GHD who stop GH replacement therapy after attaining adult height lose the acquired body composition (muscle) and gain fat[112], and adults who were treated with GH during childhood show maturational deficits compared with patients with adult-onset GHD[113]. Thus, procedures have been suggested to verify the diagnosis of GHD during transition to identify patients who qualify for continued GH replacement[114]. The extent of testing depends on the a priori likelihood of persisting GHD. This likelihood is considered high if anatomical alterations have been proved or if multiple pituitary hormone deficiency (MPHD) or a specific genetic defect is present[114]. In such cases, a low IGF1 level (<−2 SDS) suffices for diagnostic verification, whereas in other suspected cases, a low GH peak (a cut-off of <5 μg/L in standard GH stimulation tests or <16 μg/L in an arginine–GHRH stimulation test) qualifies patients for continued GH replacement according to the recommendations in adults[114].

GHD in adults

The discovery that the pituitary was the gland responsible for GH production by Harvey Cushing[12] was mainly derived from observations in adults. After the elucidation of the various metabolic effects of hGH, even Maurice Raben had stated that "GH would serve a greater function in preventing the catabolic changes of later life than in producing large individuals" (REF. 115). However, it was not until the 1980s that the GHD syndrome was recognized as an entity and treated successfully with rhGH[116].

Adult GHD can be acquired (adult-onset) or can exist from childhood (childhood-onset). Compared with healthy individuals, adult patients with GHD have an abnormal body composition (increased body fat, decreased lean body mass and low bone mineral content), metabolic abnormalities (hyperlipidaemia and impaired insulin sensitivity), a poor quality of life and social adjustment and a higher rate of cardiovascular mortality[117,118]. In addition to a strong pretest likelihood of GHD (for example, pituitary damage and/or additional pituitary hormone deficiencies), diminished GH secretion in response to testing (ITT or arginine–GHRH stimulation test) using cut-off levels lower than those used in childhood (for example, maximum GH <3 μg/L with an ITT) verifies the diagnosis[119]. Thus, in contrast to in children with a broad range of GH impairments, only severe GHD is considered for rhGH replacement in adults. Frequent sampling as a means to diagnose GHD is not considered to be useful in adults[120].

The beneficial effects of treatment with daily injected rhGH in adult patients with GHD on several clinical criteria, such as body composition, bone health, cardiovascular risk factors and quality of life and the positive influence of rhGH in reducing mortality in GHD, have been discussed in recent reviews[121–123].

Treatment with hGH

The foremost aims of GH treatment in children are the normalization of height during childhood, attainment of a timely and normal pubertal growth and the achievement of an adult height that is normal for the population and genetic target, in conjunction with normalization of other aspects (body composition, metabolism and quality of life). These aims should be reached safely and at the lowest financial expense.

Treatment with pit-hGH

Soon after pit-hGH became available and national agencies formed to collect pituitaries and to purify and distribute GH, pit-hGH treatment in children was explored systematically[124]. These patients were usually relatively old (>10 years) and had a major height deficit (<−4 SDS), often with combined pituitary hormone deficiencies. Before knowledge of normal secretion rates, pit-hGH (usually the total amount of an ampoule; for example, 4 IU) was delivered via two or three intramuscular injections per week applied with painfully thick needles by a medical professional. This treatment commonly induced catch-up growth, but normalization of height SDS was rarely attained[125]. It was not until 1983 that it was proved that daily subcutaneous pit-hGH injections not only better mimicked the normal GH plasma profile but also led to higher growth rates after transfer from intramuscular injections given three times per week[126]. This development paved the way for an independent treatment regimen administered by parents or children themselves but

also for a more variable adherence. In contrast to adults with GHD, documentation of the effects of pit-hGH on body composition has not become part of the standard follow-up procedure for children.

Treatment with recombinant hGH

In 1979, recombinant technology enabled the expression of the DNA sequence encoding hGH[127]. In the beginning, the peptide contained an additional methionine residue at the amino terminus (meth-rhGH); however, soon thereafter, rhGH could be expressed in *Escherichia coli* and in eukaryotic cell systems[127,128].

GHD. In 1985, the first meth-rhGH produced from *E. coli* was approved by the FDA in the USA for GHD in childhood. After rhGH had been marketed, long-term safety and efficacy were documented in post-marketing surveillance studies organized by the producers of various rhGH brands (for example, the National Cooperative Growth Study (NCGS) and the Kabi International Growth Study (KIGS)) or by national organizations, which assessed >200,000 treated children over ~25 years and provided a wealth of information[129,130]. The children diagnosed with idiopathic GHD in the rhGH era tended to be less short and had less severe GHD than those in the pit-hGH era[131], as discussed above. To date, thousands of children with GHD treated with rhGH have been followed to adult height. In these children — if treatment starts at about 8 years of age at a height of −3 SDS and at a dose of about 33 μg/kg body weight or 1 mg/m^2 body surface area per day subcutaneously — a normalization of adult height can be achieved in most cases[132]. However, the outcome in children with acquired GHD is still not as well documented.

Non-GHD disorders. Guided by clinical observations (pituitary gigantism) and early animal experiments, many randomized controlled trials (RCTs) evaluating the potential of rhGH to increase height have been conducted in children with growth disorders whose pathogenesis was (and still is) mostly unknown and probably not related to the GH–IGF axis, for example, Turner syndrome, short children born small for gestational age (SGA), idiopathic short stature (ISS) and short stature homeobox protein (SHOX) deficiency. In these studies, height velocity increased in the first years of treatment, and adult height gain was in the order of 1 standard deviation (6–7 cm)[133,134]. This led to registration of these diagnoses by various agencies (TABLE 1). In some disorders, abnormalities of the GH–IGF axis might have a role (for example, in chronic renal insufficiency, Prader–Willi syndrome and Noonan syndrome)[135–137]. In addition, rhGH is used for its anabolic effect (for example, in Prader–Willi syndrome[136], short bowel syndrome[138] and HIV wasting syndrome)[139]. The rhGH doses applied are usually about 1.5–2-fold higher than those used for GH replacement in GHD. Subsequently, rhGH treatment was approved for a number of growth disorders by respective regulatory authorities (varying between countries) after short-term (one or several years) efficacy and safety were documented (TABLE 1; Supplementary Box S2).

GH dosing

In the first study evaluating GH dosing, the effect of pit-hGH given intramuscularly twice weekly at a dose of 10 or 20 IU per week for up to 3 years was compared in 55 children with GHD[140]. The higher dose resulted in a height gain of 1.7, 2.7 or 3.4 cm more than that of the lower dose after 1, 2 and 3 years, respectively, and an estimated gain in adult height of ~10 cm. A more detailed study in childhood GHD during the first year, in which pit-hGH was injected three times per week at a dose of 30–100 mIU per kg body weight, showed a height velocity linear to the logarithm of the dose[141]. The positive effect of both frequency of injections and dose on growth was confirmed in a later independent RCT with rhGH[142]. The dosing of rhGH is based on body size (weight or body surface). However, dosing in non-GHD disorders reflects those used in the RCTs that led to approval and are not necessarily optimal for these indications. In adults, the

Table 1 | **Approved indications for recombinant human GH**

Year of first approval	Indication	Region
1985	Childhood GHD	USA[a], Europe and Japan
1993	Chronic renal insufficiency	USA[a], Europe and Japan
1996	Turner syndrome	USA[a], Europe and Japan
1996	Adult GHD	USA[a], Europe and Japan
1996	HIV wasting syndrome	USA[a], Europe
1997	Achondroplasia	Japan[a]
2000	Prader–Willi syndrome	USA[a], Europe and Japan
2001	SGA	USA[a], Europe and Japan
2003	ISS	USA[a]
2004	Short bowel syndrome	USA[a] and Europe
2005	GHD in transition	USA[a] and Europe
2006	*SHOX* haploinsufficiency	USA[a] and Europe
2007	Noonan syndrome	USA[a]

GHD, GH deficiency; ISS, idiopathic short stature; SGA, short children born small for gestational age. [a]Country of first approval.

Turner syndrome
A dysmorphic syndrome with short stature caused by the (partial) loss of one X chromosome in females.

Idiopathic short stature
(ISS). Refers to short stature not explained by defined causes.

Chronic renal insufficiency
A term describing a severe form of renal failure that is associated with growth failure in children.

Prader–Willi syndrome
A congenital syndrome associated with severe obesity, mental retardation and short stature (OMIM 301900).

Noonan syndrome
A dysmorphic syndrome associated with phenotypical congenital heart defects and short stature (OMIM 615355).

Short bowel syndrome
The malabsorption disorder caused by the missing of functional small intestine.

HIV wasting syndrome
The severe loss of body mass due to an infection with HIV.

GH dose is titrated upward until an IGF1 level in the upper normal range (between 0 and +2 SDS) for age is achieved[121,122].

GH Safety

In principle, the safety of a medication might be related to the characteristics of the drug and its formulation (for example, substance, impurities, dose, specific effects, induced metabolites and mode of application) or characteristics of the recipient (for example, age, sex, disorder, genetic constitution, concomitant disorders or therapy). In this section, we discuss safety concerns during and after treatment with pit-hGH or rhGH, as well as potential long-term risks.

Pit-hGH and Creutzfeldt–Jakob disease

During the early pit-hGH era, GH replacement was assumed to be safe, reporting of adverse drug reactions (ADRs) were not routine practice, and the focus was on the known effects of GH (for example, lipolysis at the injection site) or on anti-GH antibodies that were attributable to the impurity of hGH[143]. In 1984, Ray Hintz, based in Stanford, USA, reported on a 20-year-old patient who had been treated with cadaveric hGH between ages 3 and 17 years and developed a complex neurological disease characterized by ataxia and rapidly progressing dementia that led to his death within 6 months[144]. Post-mortem investigation showed the presence of the fatal spongiform encephalopathy Creutzfeldt–Jakob disease, which is caused by misfolding of a prion protein (PrP). In Creutzfeldt–Jakob disease, a pathological prion protein (PrP^{Sc}) interacts with (or 'infects') a normal PrP, leading to a cascade of protein misfolding and ultimately destruction of brain tissue[145]. If a pituitary gland that is infected with such pathological prions is used for the production of pit-hGH — which is often prepared from >1,000 pituitaries per batch — and if the PrP^{Sc} is not removed during the purification process, the pathological prion agent can be transmitted to pit-hGH recipients. The recognition of the pathological context in the case reported by Hintz[144] led to the immediate cessation of treatment with cadaveric hGH distributed by the NPA and from other (but unfortunately not all) sources in April 1985 (REF. 144). Overall, 226 cases — mainly from France, the USA and the UK, where national agencies provided the hormone — have been reported to date[1]. Although the incubation time of Creutzfeldt–Jakob disease can be up to 40 years, it seems that this epidemic is approaching its end.

Recombinant hGH

Although any medical occurrence during drug treatment is termed an adverse effect, an ADR is defined as an adverse effect that is causally related to the medication, is noxious and unintended and occurs at normal doses[146]. It is mandatory to document any medical occurrence of an adverse effect during an RCT that is designed to evaluate drug efficacy and safety. As ADRs might be rare or might occur with a time-lag, sufficient information about the safety of a drug cannot be derived from the initial clinical studies. After approval of a medication by regulatory agencies, physicians are also required to document adverse effects and to report all serious adverse effects (for example, deaths, life-threatening conditions, hospitalizations, permanent damage or birth defects) and ADRs. Nevertheless, in individuals without specific risk profiles (for example, GHD caused by malignancy) or who are not participants in specific studies, this reporting is usually not performed. In post-marketing surveillance studies that were conducted after the approval of rhGH, documenting adverse effects was commonplace, but only on a voluntary basis. Unfortunately, a few years ago, the main post-marketing surveillance studies (NCGS and KIGS) were closed, further limiting the reporting of adverse effects. Thus, valid information on the safety of GH is mostly related to serious, permanent and life-threatening ADRs.

Adverse effects during treatment. There is a wealth of articles discussing the safety of GH administration in adults and children[147,148]. Typically, during GH treatment, the ADRs that are observed are related to the induced growth process itself (for example, slipped capital femoral epiphysis and scoliosis), the water retentive quality of GH (for example, benign intracranial hypertension) or the effect of GH on glucose metabolism (for example, decreased insulin sensitivity)[147,148]. In children, the frequency of adverse effects depends on the underlying disorders being treated with GH and is lowest in idiopathic GHD or ISS (78 per 100,000 treatment years) and highest in craniopharyngioma or Prader–Willi syndrome (184 per 100,000 treatment years)[147]. Recently, the focus has been on the potential risks of mortality, cancer and cardiovascular diseases with GH. In a systematic review[149], the overall all-cause standardized mortality ratios (SMRs) were increased, but malignancy and cardiovascular SMRs were not significantly increased. However, with regard to children and adults, it can be stated with some certainty that rhGH given by the current mode, dose range and indications is remarkably safe[119,148].

Potential long-term risks. There is no convincing evidence for the induction of de novo occurrence or relapse of pre-existing malignancies by hGH treatment[149,150]. In particular, this conclusion was also reached after investigation of the literature on adults whose cancer risk is a priori increased by hGH treatment[123,151]. Nevertheless, results from a retrospective European study that assessed the safety and appropriateness of rhGH treatment in children, focusing on death and cancer as end points, had initially suggested an increased neurovascular mortality but ultimately generated inconclusive results[150,151]. Thus, the controversy about how to best document the long-term safety of rhGH continues[152]. However, as a physician's duty is 'first, do no harm', it is prudent to be aware of Douglas Frasier's comment with reference to the end of the pituitary hGH era: "I believe that, first and foremost, we must maintain the healthy respect for the law of unintended consequences" (REF. 153).

Future developments

New information and innovations on the physiology and pathophysiology of the GH–IGF axis will continue to emerge and develop from basic research, animal

Creutzfeldt–Jakob disease
A prion-transmitted degenerative encephalopathy.

experiments and studies in humans. Evidently, some future developments will continue along the paths already taken, whereas others that appear distant today or that are currently unknown might eventually find their way into clinical practice. In this section, we discuss some potential future developments in the diagnosis and treatment of growth disorders and other conditions.

Next-generation sequencing

The rapid technological advances in diagnostic genetic tools, including next-generation sequencing technologies such as whole genome and exome sequencing, have enabled the discovery of many genetic disorders causing GHD in short children that had been previously stratified incorrectly under the general terms of SGA and ISS. For example, children carrying heterozygous mutations or deletions in *SHOX* and its enhancer region, *NPR2* mutations or *ACAN* mutations have no disturbance in the GH–IGF axis, but their presentation as ISS or SGA (these patients generally have normal body proportions) renders them potential candidates for hGH treatment[105]. However, the frequency of each of these gene defects in short children who were previously considered ISS or SGA is probably close to 1–2%[105]. There is no doubt that other genetic disorders will be uncovered over the next decades.

This development will have implications for the use of rhGH to treat growth disorders, particularly for the broad diagnostic criteria of SGA and ISS. Part of the large variability in the growth response to rhGH treatment for these indications might be related to differences in genetic aetiology. As genetic screening of short children in the future will be facilitated by its increasing availability and decreasing expense, it will be feasible to perform genetic testing of children who have been treated with GH under the indications of SGA and ISS. This will enable attainment of a better insight of GH efficacy and safety in children with specific genetic disorders.

New therapeutic approaches

New therapeutic approaches for disorders of the GH–IGF axis and various forms of short stature are likely to originate from a novel understanding of their pathophysiology. However, new therapeutic approaches within the frame of our current knowledge have already emerged. These approaches include optimizing treatment with conventional rhGH, long-acting hGH, the prospect of gene therapy and GH–GHR antagonists, which are discussed briefly.

Optimal use of daily rhGH. The objective of GH replacement and therapy is to optimize and individualize treatment with respect to growth and other effects of GH and to aim for the most cost-effective and safe treatment approach. With regards to optimizing and individualizing GH therapy in terms of linear growth in children, prediction algorithms and IGF-guided therapies have been proposed by several groups[3]. Prediction models are mathematical algorithms derived from data on variables associated with the growth response obtained from large cohorts and attempt to describe the predicted growth response of an individual with a certain disorder (GHD, Turner syndrome, SGA or ISS) during a specific growth phase (prepubertal, pubertal or total growth from start of treatment to adult height), with the smallest possible error. The predictors chosen before treatment onset vary according to the model and might include easily available anthropometric parameters (for example, age, distance between height SDS and MPH SDS, bone age or early postnatal weight) and biochemical parameters (levels of GH peak after stimulation tests, IGF1, IGFBP3, leptin or markers of bone resorption) as well as variables related to the genetic constitution of the individual[3,154,155] (TABLE 2). The observation that a common *GHR* polymorphism (for example, *GHRd3*) can be associated with a greater response to GH[62] has stimulated investigations in search of other variables related to the individual's genomic constitution and changes in gene expression as predictors of the response to GH[155].

An alternative approach to optimize the GH dose to the patient's sensitivity to GH is to guide the GH dose to achieve a serum IGF1 target with treatment[156]. A GH dose that maintains serum levels of IGF1 at 0 SDS seems to be the most cost-effective[157]. Further developments in combination with easily accessible computer systems have the potential to optimize outcome variables and might also lead to a more economical use of rhGH[158]. In addition, an important method to optimize the effect of rhGH treatment is to closely monitor adherence to GH injections and, if found suboptimal, use techniques to improve it (for example, patient and/or parent education, medication reminder systems and motivational interviewing techniques)[159].

Long-acting GH. Attempts to administer GH by alternative routes (intranasal, inhaled or transdermal) have not yet found their way into clinical practice. This also applies to treating GHD states with GHRH, oral ghrelin analogues and potentially other GH stimulatory substances or their modifications. However, in the past decade, the idea of avoiding daily injections by using long-acting (1–2 weeks) GH (LAGH) preparations has gained much attention[160]. The main argument for the need for such preparations is the claim that less frequent injections would be patient-friendly, reduce non-adherence and subsequently improve growth outcome[159].

The approaches taken for the development of LAGH preparations vary and can influence the pharmacokinetics and/or the pharmacodynamics of rhGH. To date, LAGH preparations have been developed using various approaches to prolong GH action, including the following: forming emulsions (using gelatine and triglycerides); GH encapsulation (using degradable microspheres); GH pegylation; GH conjugation (to albumin or amino acid 'tails'); and GH fusion proteins (by means of linking an inert peptide with rhGH at a region that does not interact with the GHR)[160]. The rhGH molecule itself might be unchanged or structurally modified. Different phases of clinical research studies are presently ongoing to evaluate such preparations[160]. It can be assumed that, if noninferiority compared with daily rhGH injections is shown, and if approved by regulatory authorities, LAGH

preparations will be used in all current indications for rhGH therapy. However, it remains an open question whether LAGH preparations will provide a true benefit for patients and will have the same safety profile as current daily rhGH.

The prospect of gene therapy. Gene therapy, a technique to replace, edit, silence or inhibit a pathological gene mutation, offers new approaches to treatment. There have been developments at the level of basic research regarding a cure for some forms of GHD. Using in vitro systems, mouse stem cells can be transformed into hormone-producing pituitary cells[161]. In IGHD type II, the misspliced 17.5 kDa GH isoform exerts a dominant-negative effect on the secretion of normal GH[43]. Its accumulation in the cytosol of somatotrophs can lead to other pituitary hormone deficiencies, a process that cannot be prevented by treatment with rhGH. Experiments with rat pituitary cells have shown that by modulation of the *GH1* splicing pattern, an increase in the normal 22 kDa GH isoform can be achieved[162].

GH–GHR antagonists. The artificial substitution of a single amino acid residue (Gly120Lys; glycine substituted for lysine at position 120) in hGH was shown to transform the structure of the second GHR binding site such that effective receptor binding was prevented[4]. The subsequently developed GH analogue pegvisomant (somavert) was shown to be a GHR antagonist in animal models and humans and has become part of the standard treatment (by means of daily injections) for acromegaly[163]. The effect observed in rodents of a long-acting GHR antagonist, with amino acid changes in the GH binding domain and fused to GHBP, will probably be evaluated for its efficacy and safety in humans with poorly controlled acromegaly[164]. Using similar or novel approaches, antagonists, superagonists or even analogues with cell-specific actions of GH, GHRH, somatostatin, ghrelin and other components of the GH system might be designed in the future.

Ageing and malignancies

As GH secretion and GH-dependent IGF parameters in blood constantly and progressively decrease with ageing after having peaked during adolescence, the term 'somatopause' was coined to describe the situation in elderly individuals. GHD and ageing are both associated with a decreased anabolic state, leading to a decrease in lean body mass (sarcopenia) and bone mass and an increase in body fat[165]. Similar to adults with GHD,

Table 2 | **Components and characteristics of growth prediction models for the first 1–2 years on GH**

Diagnosis	Prediction duration (response variable)	GH dose	Predictors	*n* (R^2) [error SD]
Ranke*[178] *(KIGS, Europe), 1999				
GHD	First year (cm/year)	Variable	• Max test GH • GH dose • Birthweight (SDS) • Age (years) • HT–MPH (SDS) • WT	593 (0.61) [1.5 cm/year]
Kriström*[184] *(Gothenburg, Sweden), 1997				
• GHD • ISS • SGA	24 months (ΔHT [SDS])	Fixed (33 μg/kg per day)	• IGF1 (SDS) • Max test GH • ΔIGF1 at 3 months on GH (SDS) • ΔWT (SDS) a year before GH • Age (years) • WT at age 1 year	69 (0.58) [0.18 SDS]
Wikland*[185] *(Gothenburg, Sweden), 2000				
• GHD • ISS • SGA	24 months (ΔHT [SDS])	Fixed (33 μg/kg per day)	• Max GH during 24 h (mU/l) • Age (years) • HT (SDS) • WT (SDS) • Sex • BW (SDS) • HV (SDS) (second year) • HV (SDS) (year before)	269 (0.45) [0.19 SDS]
Schönau*[186] *(Cologne, Germany), 2001				
• GHD • TS	First year (cm/year)	Fixed (23 μg/kg per day)	• (Age–BA): BA (years/year) • IGF1 (μg/l) • uDPD (24 h, at 1 month on GH) • HV (cm/year) (0–3 months on GH)	58 (0.89) [2.0 cm/year]

Based on the empirically observed growth to recombinant human growth hormone (rhGH) of a large cohort with a defined growth disorder (diagnosis), mathematical algorithms (prediction models) are derived that allow the calculation of the most likely growth response (for example, cm/year) and its error during a growth phase (for example, the first year) based on a patient's characteristics (for example, height and age) and the chosen GH dose at start or a short time thereafter. The prediction algorithms (prediction model) should explain as much of the variability of the response as possible ($R^2 = 1.0 = 100\%$) with the least possible error. BA, bone age; BW, birthweight; GH, growth hormone; GHD, growth hormone deficiency; HT, height; HV, height velocity; ISS, idiopathic short stature; MPH, midparental height; SGA, small for gestational age; TS, Turner syndrome; uDPD, urinary deoxypyridinoline; WT, weight.

treatment with hGH in healthy elderly individuals was shown to partly revert sarcopenia[166]. For this reason, the idea of using GH as the anti-ageing drug inspired the research of many scientists in the 1990s.

However, by contrast, the potentially beneficial effect of increased GH–IGF axis activity has been challenged in the past few years. Indeed, knockout mouse models of GHD (Ames and Snell mice), GH insensitivity (*Ghr*$^{-/-}$ and Laron mice) or IGF1 insensitivity (heterozygous deletions of *Igf1r*) live longer than wild-type mice[167]. There are also suggestions that patients with *GHR* defects have a lower cancer risk and a greater than normal life expectancy[168]. Epidemiological studies in centenarians measuring components of the GH–IGF axis in blood suggest an association between IGF1 sensitivity and longevity in men[169]. Thus, the role of endocrine components in human ageing is of great complexity and requires further study. However, the concept of tissue targeting using newly designed agonists or antagonists of the GH–IGF axis to achieve specific effects remains challenging.

Conclusions

After the role of GH as a pituitary peptide hormone with a major role in growth and metabolism had been established by clinical observations, by animal experiments and by biochemical investigations during the first half of the 20th century, treatment of GHD using pit-hGH in humans began. This era coincided with the discovery of IGFs and their complex system. Subsequent advances in molecular genetics led to the development of rhGH and to the detailed understanding of components of the GH–IGF axis and their genetic regulation, as well as associated growth disorders in humans and in animal models. Along with new technologies such as next-generation sequencing and genetic engineering, this process is presently ongoing at a rapid pace. The development of therapeutic GH agonists and antagonists holds the promise of a more efficacious treatment for currently known disorders of the GH–IGF axis and the exploitation of these agents in new therapeutic areas such as metabolism and malignancies.

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Competing interests
M.B.R. declares that he is a member of the Pfizer advisory board for iGRO and has received honoraria for speaking from Pfizer and Sandoz. J.M.W. declares that he is a member of the Merck advisory board and has received honoraria for speaking from Sandoz, Merck-Serono, Pfizer, Versartis, Eli Lilly, Novo Nordisk and JCR.

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