Pediatric Endocrinology Reviews
Diabetes Nutrition Metabolism Genetics
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60 Years of hGH

Chief Guest Editor:
Adda Grimberg, MD

Co-editors:
Paulo Ferrez Collett-Solberg, MD, PhD, Raoul Rooman, MD, PhD,
Toshiaki Tanaka, MD, PhD

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Foreword

In August 1958, Dr. Maurice Raben published the first report of a human receiving human growth hormone (HGH) treatment; the patient had GH deficiency, and the HGH had been obtained from pituitary extracts of donor cadavers (Raben MS. J Clin Endocrinol Metab 1958;18(8):901-903. Commemorating the 60th anniversary of this landmark event, we sought to compile a group of papers that convey the broad narrative – both historically and scientifically – of the story of HGH.

Also reflective of the HGH narrative over the past 60 years, the collection beautifully represents an international collaboration. Not only have individuals from all over the world contributed new knowledge and insights to the HGH story, but much of the important work has been accomplished via international multi-center studies, debate and guidelines. This is perhaps most evident in the multiple international studies of HGH safety – both post-marketing surveillance registries and international consortiums examining safety issues – where sufficient study power requires cooperation beyond national borders.

Our HGH historical narrative begins with the Bible and carries us to the present, with an eye to the future. The tremendous progress made in some aspects is both heartening and humbling – such as elucidation of the hormones, their receptors and mechanisms of action; the recognition of both endocrine and autocrine/paracrine regulators of growth; and the identification of an ever-growing list of genetic conditions that impair human growth. However, some challenges have persisted virtually unaltered through the decades – such as limitations of provocative testing and lack of assay harmonization making it hard to define who truly has GH deficiency; paucity of quality evidence on psychosocial effects fueling the on-going debate over whether children with idiopathic short stature should be treated with HGH; and if HGH treatment is extended beyond GH deficiency, what are the appropriate patient characteristics, outcome measures, and ethical and resource allocation considerations to provide a favorable benefit-risk assessment. Although we have come far, HGH clinical use is still too young for determination of any long-term, post-treatment effects (i.e. into late adulthood), and thus, continued international collaboration remains crucial.

We thank the many talented clinicians and scientists who authored the stories in our HGH narrative, and we look forward to continuing to work together with them and colleagues the world over as the HGH field continues to develop.

Adda Grimberg, MD **Paulo Ferrez Collett-Solberg, MD, PhD** **Raoul Rooman, MD, PhD** **Toshiaki Tanaka, MD, PhD**

Footnote:

To maintain consistency throughout the issue, the following abbreviations have been used:

GH = endogenous hormone (+/- exogenous)

hGH = human GH (pituitary)

rhGH = recombinant human GH

metGH = recombinant methionine-GH

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Growth Hormone Discovery and Structure

Mat Buchman*, BS, Stephen Bell*, BA, BS, John J. Kopchick, PhD

Heritage College of Osteopathic Medicine, Department of Biomedical Sciences, Ohio University, Athens, Ohio, USA

Corresponding author: John J. Kopchick, PhD, Distinguished Professor & Goll-Ohio Eminent Scholar, Edison Biotechnology Institute, Konneker Research Laboratories, Water Tower Drive, Ohio University, Athens, Ohio 4570, USA, E-mail: kopchick@ohio.edu

*These authors contributed equally to the manuscript and should be considered first author

Abstract

The purpose of this review is to describe and document the discovery of growth hormone (GH) and various activities associated with it. Crucial to this discourse will be a chronicle of results related to the structure of GH. Many individuals were instrumental in the early and current work. Throughout the review we present glimpses into their scientific lives as it affects the evolution of GH's story. We realize that we have not presented a comprehensive review of GH's history and its current and future status, and apologize for the omission of many individuals who contributed to this story.

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***Key words**: Growth hormone, History, Discovery, Structure, Somatomedin, Acromegaly, Function, Action*

The Early Years

Meekly staring down their Philistine enemies, the ancient Israelite army was not at all eager to confront them. Twice a day the Philistine champion Goliath would come out and mock the Israelite army for their cowardice, goading them into sending out their own champion to face him. Described in some texts as being nine feet tall, the giant Goliath was a menacing presence. All who were at the battlefield were fearful. Everyone, that is, except for a young shepherd boy named David who was there to feed his brothers. Unafraid and filled with courage, the young boy volunteered to fight against Goliath. Collecting the smoothest stones he could find, he went out with his sling and confronted the giant. Goliath laughed in his face. Yet his laughter was short lived. Letting loose a single stone from his sling, the rock zipped quickly and firmly into Goliaths forehead. Acting swiftly to finish the job, David took Goliath's sword and lopped off his head. The Israelite army, inspired by David, picked up their arms and routed the Philistine army in what becomes a glorious day (1 Samuel: 17, New International Version Bible).

Looking at this story in a modern context, Goliath's height leads to a question: Did he have the disease we know today as acromegaly, a condition in which pituitary tumors cause excessive growth hormone (GH) release leading to increased growth and accompanying complications? Or was it some sort of hereditary gigantism? All of his brothers as well as his children are described as being great in stature; thus, there may well be a genetic influence here. If Goliath did have a GH secreting, large pituitary adenoma, it may well have compressed the optic chiasm reducing peripheral field vision, allowing David to 'sneak-up' on him and sling the stone. Whether this description of David and Goliath is true or a legend is not known; however the condition of acromegaly was one of the first described endocrine diseases.

The history of acromegaly continues to the Roman Empire. The emperor, Maximinus Thrax, using modern measurements

was described as being 8 feet six inches in height and having the ability to wear his wife's bracelet as a ring. This description given could be characteristic for someone with acromegaly. Looking at his bust on Roman coins and statues, we can see features that are diagnostic of someone with acromegaly. The thick skin of his forehead and enlarged nose are drastic departures from other Roman statues and hints at characteristics of patients with acromegaly (1).

Before the term GH was defined, several individuals were aware of a disease which affected growth. The French Neurologist, Pierre Marie, in 1886 would become the first person to use the term "Acromegaly" in his paper *"Sur deux cas d'acromégalie; hypertrophie singulière non congénitale des extrémités supérieures, inférieures et céphalique"* (2). In English, this translates to "On two cases of acromegaly; non-congenital singular hypertrophy of the upper, lower and cephalic extremities" (google translate). Pierre Marie, thus, gives a detailed description of patients with acromegaly and provides the term still in use today.

Indeed, descriptions of patients with acromegaly have been in medical literature for centuries. For example, Dutch surgeon and occult skeptic, Johannes Weir in 1567 described a patient with acromegalic symptoms (3). Some attempts to name this disease were also made before the term acromegaly was settled upon. In 1864 Italian doctor Andrea Verga used the term "prosopo-ectasia", or face widening, to describe a patient that had a sellar tumor, the size of a walnut, that displaced or impacted the optic nerves (4). In 1887 Minkowski also noted that in all cases of acromegaly that he had observed, there had been pituitary enlargement (5).

In June 1905, Ernest Starling, a professor of physiology at University College London, coined the word "hormone" (6). Like many English words, he derived this new word from the Greek term "hormon" which literally means 'to set in motion'. Professor Starling was the first to use the word, hormone, during an invited Croonian Lecture at the Royal College of Physicians in London (7). He postulated that there must be some sort of chemical messengers within the human body that would help regulate biological functions. It was not until Starling's lecture that the scientific community had a specific word for those messengers.

Growth Hormone – Who 'Coined' the Name?

Harvey Cushing

Further elucidations about the function and purpose of the pituitary came from American master surgeon Harvey Cushing. He was born on April 8, 1869 in Cleveland, Ohio as the 10th child of Elizabeth Williams and Henry Cushing. In 1891 he graduated from Yale University and went on to earn his M.D. from Harvard Medical School in 1895. His contributions to the GH field were invaluable. In a 1912 book *The Pituitary Body and its Disorders*, he was the first to use the terms "hypo and hyperpituitarism". He noted that short stature or increased growth were both associated with structural changes in the pituitary. This led him to postulate that there was a particular "hormone of growth" (pp. 237, 256) secreted by the pituitary, however, no description of the chemical nature of this hormone was established (8). Later, his 1932 paper, *The Basophil Adenomas of the Pituitary Body and Their Clinical Manifestations (Pituitary Basophilism)*, was the first to hypothesize that pituitary adenomas led to the excess secretion of GH (9). Cushing is especially well known for his intense work on pituitary adenomas which led to a disease named in his honor; Cushing's disease (10).

Herbert McLean Evans

Experiments involved in the isolation and characterization of the chemical messengers (hormones) affecting growth followed. Prior to the isolation and chemical identification of specific hormones, they were instead named after the properties with which they were associated. For example, as stated above, Cushing postulated a 'hormone of growth'. More information about this 'hormone of growth' would come from famed scientist Herbert Mclean Evans (**figure 1a**). Born in Modesto, California in 1882 he would go on to attend Johns Hopkins School of Medicine. Graduating in 1908, he found himself more interested in research than clinical work. This was much to the chagrin of his father who practiced surgery back home in California. Instead he took a job doing research for anatomical researcher Franklin P. Mall at Johns Hopkins (11). His decision to pursue research over clinical practice would prove fateful. In 1921, now back in his native California after being offered the job as Chair of the Department of Anatomy at Berkeley, Evans and Long noticed that extracts from the anterior lobe of the bovine pituitary gland, when injected into rats, promoted growth (12). However, the chemical entity that caused this 'extra growth' was unknown.

Evans is known as the founder of GH, however, the earliest reference that we could find that specifically calls GH by its name comes from a 1932 manuscript by Dr. William Engelbach in which he attempted to treat children with short stature with what he calls **growth hormone**. In this experiment he did report some increased growth in one of his patients in which he was using partially purified bovine GH (bGH) (13). However, it was shown later that bGH is ineffective at promoting human growth (14). Thus, between Evans' discovery of a growth promoting substance in 1921 and Engelbach's naming of GH in 1932, we could not find another publication in which the term 'growth hormone' is used.

Growth Hormone – Structure, Function, and Actions

Bernardo Alberto Houssay

GH has many other important properties besides growth promotion. In describing acromegaly, Pierre Marie noted elevated amounts of glucose in the urine of patients with acromegaly (2). Diabetes was, and is, a common feature in such patients. Inspired by the discovery of insulin by Banting and Macleod in 1921, the Argentinian physiologist, Dr. Bernardo Alberto Houssay (**figure 1c**), began his work on assessing the effect that the hypophysis had on glucose metabolism. Born April 10, 1887 to Dr. Albert and Clara Houssay, he was one of 8 children. Bernardo was a bright young man who went to college at the age of 14 and graduated when he was 17. As a member of the Department of Physiology at the University of Buenos Aires he began his research on the hypophysis which culminated as his M.D. thesis for which he won a university award. He would lead the University of Buenos Aires Institute of Physiology starting in 1919 until being 'kicked out' in 1943 by the government for his pro-democratic viewpoints (15).

In his seminal experiments, he used two animal models; dogs and a common Argentinian toad called *Bufo marinus*. Upon removing the anterior lobe of the pituitary from these animals, they would become hypoglycemic and die when treated with what would be considered for unhypophysectomized animals a small amount of insulin. Thus, it was shown that some yet to be isolated hormone of the pituitary had an antagonistic relationship with insulin. He published his results in a 1931 paper (16) that unfortunately we could not access but it is referenced in The New England Journal of Medicine (17) . Sixteen years later in 1947, he would jointly share the Nobel Prize with Czech scientists Carl and Gerty Cori for their work related to the metabolism of glucose and glycogen within the body.

Choh Hao Li

Sometimes the student will work to outshine the master. The case can be made that Dr. Choh Hao Li (**figure 1b**) did just that. Born in Canton, China in 1913 as the son of a successful industrialist, Li would earn his Bachelor of Science degree from the University of Nanking in 1933. Afterwards he would enter the Ph.D. program in organic chemistry at the University of California, Berkeley. It was there that previously discussed scientist, Herbert Evans, discovered Li and hired him to work in his lab. His work would culminate in a storied career that will be discussed in greater detail later (18). In 1944 Dr. Choh Hao Li along with Evans, Simpson, and Marx isolated what we now know today to be bGH (19,20). This was determined to be a 44.2 kDa protein (note: GH is a 22kDa protein) that did not share any of the other effects that were derived from anterior lobe pituitary extractions including stimulated ovulation and early onset sexual development in rats injected with the extract (21). Injection studies resumed in 1947 by Li and Evans with bGH in a woman who was described as a "hypopituitary dwarf". When no growth occurred, the theory of species specificity of GH began (22). More data by Li and colleagues concerning the structure and activity of GH will be presented later in this review.

Zvi Laron

Lack of growth in certain patients was not limited to incidents of hypopituitarism. A curious case unfolded in 1958 in the clinic of Dr. Zvi Laron. Zvi was born to a Jewish family in the Czernowitz-Bukovina region of northern Romania in 1927. This region once was a part of the Austro-Hungarian Empire until 1918. Zvi was one of many individuals who were sent to the concentration camps after the invasion of the USSR by Nazi Germany. He was able to survive the war as a forced laborer in a factory founded by his uncle Siegfried Sami Jagendorf. The factory was founded with the purpose of providing cover to Jewish deportees who otherwise would have been executed by the Nazis. After the war, he made his way to the state which would become Israel. He attended the Hebrew University Medical School where he received his MD. In 1958 he helped found the Institute for Pediatric and Adolescent Endocrinology in the Beilinson Medical Center, which was associated with Tel Aviv University.

It was in this newly founded institute in 1958 that three young patients aged 3.5, 1.5, and a newborn baby were brought to his attention. Among them were two boys and one girl. Each patient was suffering from idiopathic short stature and each was completely insensitive to hGH treatment. It was not until 1966 with the advent of new hGH assays that they were able to examine the circulating levels of GH in these and similar patients. Much to their surprise each child had elevated serum levels of GH typical of those found in patients with acromegaly. Two hypotheses were put forward; either the structure of the GH produced by these patients was defective, or there was a receptor defect of some sort making them resistant or insensitive to GH. In 1984, they were able to definitively answer this question after taking two liver biopsies from volunteering patients. They showed that hGH does not bind to the liver GH receptors (GHR) of these patients. Cloning of the GHR in 1987 and its characterization in 1989 definitively showed that the problem laid with the GHR and not with the GH (23). The subject of Laron Syndrome patients will be described in another chapter in this volume.

Activities of "Gland" Extracts

Potential hormone therapies to treat certain maladies or improve performance have been attempted for years. In 1889 baseball pitcher, Pud Galvin, of the Pittsburgh Allegheny's pitched a two hit shutout against the Boston Barnbeaters

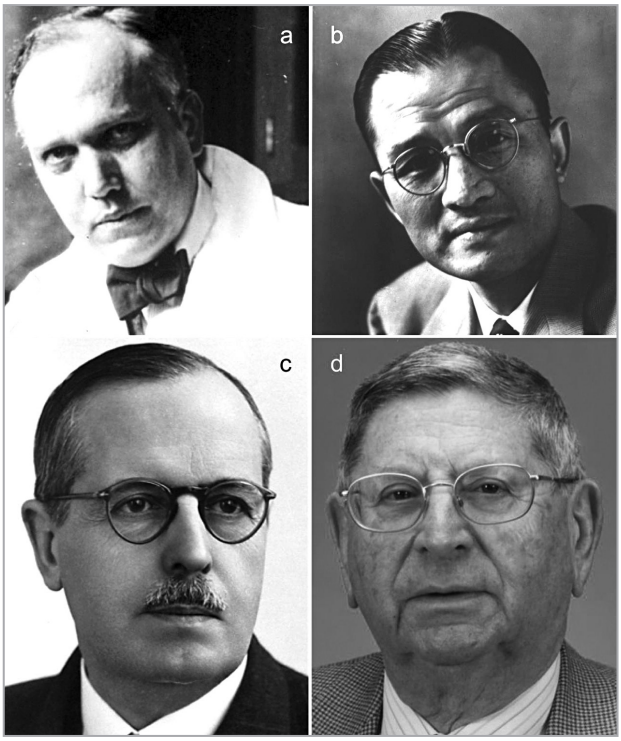

Figure 1. Pioneers in the GH field
***a**. Herbert Evans*
***b**. Choh Hao Li*
***c**. Bernardo Houssay*
***d**. Zvi Laron*

after injecting himself with an extract of hamster and dog testicles (24). Similarly, Dr. Charles-Édouard Brown-Séquard was a famous advocate of using hormone therapy to try to restore his own vitality. It was his belief that one of the causes of aging in men was the "gradually diminishing action of the spermatic glands". To remedy this problem, he would inject himself with a mixture of guinea pig or dog testis extracts. At age 72, with his strength failing him, he found that after injecting himself with his extract, he "regained at least all the strength I possessed a good many years ago" (25).

While many were skeptical about his claims, the use of endocrine organ extracts to treat disease did eventually find greater and more legitimate use. In 1958 Dr. Maurice Raben began treating GH deficient (D) patients using GH purified from pituitary extracts of donor cadavers (26). This would become the standard practice for the next ~30 years.

The disease today known as Creuzfeldt-Jakob is named after two different scientists; Hans-Gerhard Creutzfeldt and Alfons Jakob. Both were the students of another famous scientist who bears the name of a disease, Alois Alzheimer. In 1920 Creuzfeldt, a neurologist by training, published his study of the disease with a patient named "Bertha E." This woman was suffering from tremors, spastic movements, altered gait, and dementia. The collection of symptoms she displayed in his mind pointed to a brand-new disease. In 1921 pathologist Alfons Jakob came forward with three similar cases that he reported to the German Neurological Society. All of his patients displayed similar symptoms to Creuzfeldt's and died within a year of displaying symptoms. Autopsy results showed diffuse neuronal degeneration as well as glial cell proliferation in the cerebral cortex and basal ganglia. He would term the disease "spastic pseudo sclerosis with disseminated encephalomyelopathy" (27). In 1922 fellow scientist Wolfgang Spielmeyer gave it the name Creuzfeldt-Jakob (28).

In 1985, a group of clinicians published in the New England Journal of Medicine a new warning about a potential epidemic of Creuzfeldt-Jakob disease caused by hGH therapy using hGH derived from human cadavers (29). Creuzfeldt-Jakob disease is now known to be caused by prion like particles and result in encephalopathy. Some of the patients who had received GH collected from human cadavers began showing symptoms of this disease. For this reason, in 1985, the use of human cadaver derived GH was immediately ceased. Thankfully, in the same year recombinant methionyl-human growth hormone (metGH) was approved for use. By inserting the hGH gene (cDNA) into E.coli, synthetic recombinant metGH was produced that did not possess contaminants including pathogenic prions (30). Later, metGH as well as recombinant human growth hormone (rhGH, recombinant GH that did not possess an extra methionine) would receive approval for a wide variety of conditions in addition to childhood GH deficiency. These include chronic kidney disease (US 1993), Turner syndrome (US 1996), AIDS wasting syndrome (US 1996), adult GH deficiency (US 1996), achondroplasia (Japan 1997), Prader-Willi syndrome (US 2000), small for gestational age or "SGA" (US 2001), idiopathic short stature or "ISS" (US 2003), small bowel syndrome (US 2004), and Noonan's syndrome (US 2007).

The Somatomedin Hypothesis

The idea that GH does not act independently to effect target tissue was postulated over 60 years ago. Daughaday made observations that SO4 incorporation into cartilage was reduced in hypophysectomized rats; however, upon injection of bGH into the hypophysectomized rats, SO4 incorporation was restored (31). He postulated that GH induced a sulphation factor. Interestingly, when results were replicated *in vitro* by placing bGH onto rat costal cartilage, no growth effect was seen (32). Later, isolated chondrocytes were treated with normal serum as well as serum from individuals with hypopituitarism. Normal serum caused as great as a 100-fold increase in thymidine incorporation when compared to hypopituitary serum (33). This GH-induced circulating factor was deemed "sulphation factor" for its ability to stimulate SO4 incorporation into cartilage (34).

The designation as sulphation factor was short-lived after this factor was shown to have broader impacts such as insulin-like actions in the rat diaphragm assay (34), proline to hydroxyproline conversion in rat cartilage (35), and the ability to stimulate HeLa cell growth (36). After careful consideration, Daughaday proposed renaming sulphation factor "somatomedin." This came from "somato" connoting a relationship to somatotropin and "medin" to indicate that it is an intermediary in somatotropin action (37).

The Swedish clinician, Dr. Olle Isakson, challenged the notion that circulating somatomedin was uniquely responsible for the effects of longitudinal bone growth by GH. In 1982, he showed that local administration of GH *in vivo* resulted in bone growth. Specifically, when GH was injected into the leg of a rat, he and his colleagues observed growth in that leg while the contralateral leg experienced minimal growth suggesting that endocrine somatomedin was not responsible for bone growth. When anti-somatomedin serum was added to the leg injected with GH, no growth was seen indicating that GH was able to cause growth by locally inducing somatomedin (38). Thus, GH stimulated somatomedin could act systemically as well as locally. Later, this concept would be referred to, respectively, as the endocrine versus the paracrine/autocrine effect.

In a parallel and at the time seemingly unrelated paradigm, a non-suppressible insulin-like activity (NSILA) was described by Dr. E R Froesch. Despite removing insulin's action by mixing anti-insulin serum with normal human serum, the insulin-depleted serum was able to cause glucose uptake by adipose tissue *in vitro* (39). NSILA was determined by Humbel *et al*. to be IGF-1 after its amino acid sequence was deduced (40). Shortly thereafter, in the early 1980's, insulin-like growth factor 1 was determined to be the primary somatomedin (Somatomedin C) mediating the effects of GH (41).

In 1985, Dr. Howard Green proposed a change to this paradigm. His theory, coined the "dual-effector hypothesis," referred to the distinct functions of GH and IGF-1 in triggering tissue growth by promoting both cellular differentiation and clonal expansion of the differentiated cells (42). According to the dual effector hypothesis, GH and IGF-1 are dependent on one another for proper growth. Specifically, tissue growth occurs by GH promoting differentiation of precursor cells followed by the clonal expansion boosted by IGF-1. This idea was supported by studies where GH could directly differentiate precursor cell types such as preadipocytes whereas IGF-1 could not (43). Moreover, IGF-1 was able to selectively expand cultured fibroblasts whereas GH could not (44).

In 2001, the question arose and was answered as to what specific contribution GH or IGF-1 have on mouse growth. In this study, GHR -/-, IGF-1 -/-, and double-knockout mice were used and provided new information for the evolution of the somatomedin hypothesis (45). They concluded that 35% of growth was attained via IGF-1 while GH contributed 14%, and their combined effects accounted for 34% of growth (only 17% of growth was not associated with the GH/IGF-1 axis). Thus, GH and IGF-1 had both unique and overlapping growth related activities. To further assess the function of endocrine IGF-1, a liver-specific IGF-1 -/- mouse was produced. These mice had circulating IGF-1 levels that were 25% of WT controls. Interestingly, there was no abnormal growth phenotype observed in these mice up until 6 weeks of age (46). Recently, liver-specific GHR -/- mice have been generated. Low serum IGF-1 levels were shown along with a corresponding increase in serum GH. The low endocrine IGF-1 levels did result in diminished body size of these mice later in life (47). Possibly, the lack of IGF-1 negative feedback of GH at the pituitary resulted in increased circulating GH that may increase auto/paracrine effects of IGF-1 for growth.

GH Structure

Dr. Choh Hao Li was perhaps the most prolific scientist in the GH field. His list of publications is daunting numbering over 1,100 along with 10 honorary degrees (18). As one reviews his publication list, it is easy to see that he was 'passionate' about the structure and activities of GH. Li was not only instrumental in the isolation and purification of GH from various species (human, monkey, whale, sheep, cattle, and pig) but also in elucidating its amino acid composition in 1962 (**figure 2a**), its primary structure in 1971 (**figure 2b**), and its chemical synthesis in the 1970-1980s (48-51). To deduce the amino acid sequence of hGH, Li studied enzymatically generated fragments. From these studies, it was determined that hGH was indeed a single polypeptide (52) and that the carboxyl and amino terminal amino acids were Phe and Phe-Pro Thr-Ile-Pro-Leu-Ser-Arg, respectively (53,54). Further studies of hGH fragments derived by cleavage with trypsin, chymotrypsin, and pepsin (over 70 fragments) provided data for the elucidation of its final amino acid sequence (**figure 2a**) and revealed that the entire GH molecule was not necessary for its full biological activity as assessed by tibial width and pigeon crop sac stimulation assays (55,56).

Next Li focused on the chemical synthesis of hGH by producing various individual fragments of the protein (57-59) and assessing their biological activities (60). In 1970, Li and Dr. Donald Yamashiro reported the full synthesis of hGH; however, the peptide they synthesized was only 188 amino acids in length and retained only 10% of the growth promoting activity of native GH (61). Subsequently, Li and his colleagues shifted their focus to synthesizing fragments of GH, the first 54 amino acids of the amino terminal, exhibited approximately 7% of native GH activity (62). However, many others attempted to synthesize active fragments of GH. For example, Chillemi *et al* synthesized several peptides from the middle of hGH including 87-123 and 124-155, which showed mild growth effects in the rat tibia assay (63). Li also synthesized a fragment, 95-136,

from the middle of hGH, and achieved similar results in rat tibia assays as Chillemi *et al*. (64). Peptides of the carboxyl terminal were synthesized by Li as well as Bornstein *et al*., specifically, peptide 172-191 that was shown to possess potent hyperglycemic activity in rats (65). Eventually, Li was successful in synthesizing two large fragments of hGH that could be non-covalently combined and had similar hGH radioreceptor-membrane binding activity to that of pituitary-isolated hGH (66). Despite early momentum in GH structure/function studies, he was never able to achieve total chemical synthesis of biologically active hGH.

Because of the difficulty in synthesizing large proteins, many began searching for an "active core," or some conserved portion of the GH molecule that is responsible for its biological activities in all species (55). Discovery of an active core would make chemical synthesis of a therapeutic agent much more efficient by reducing the number of amino acids required for full biological activity (67). Interestingly, in 1977, Sonenberg and Levine showed that tryptic digestion of bGH resulted in several active fragments. They observed that fragment 96-133 decreased blood urea nitrogen and increased insulin resistance in humans with hypopituitarism (**figure 2c**) (68). Indeed, several fragments were shown to retain many of the properties of intact hGH. Of these fragments, many that were active came from the N-terminal [1-133] of the molecule whereas the fragments from the C-terminal [134-191] were largely inactive (69). The bioactivity measured by weight gain, glucose oxidation, lactogenic properties, receptor binding, tibial width increase, etc. of over 90 fragments and recombinant forms has been compiled. As such, the reader is referred to

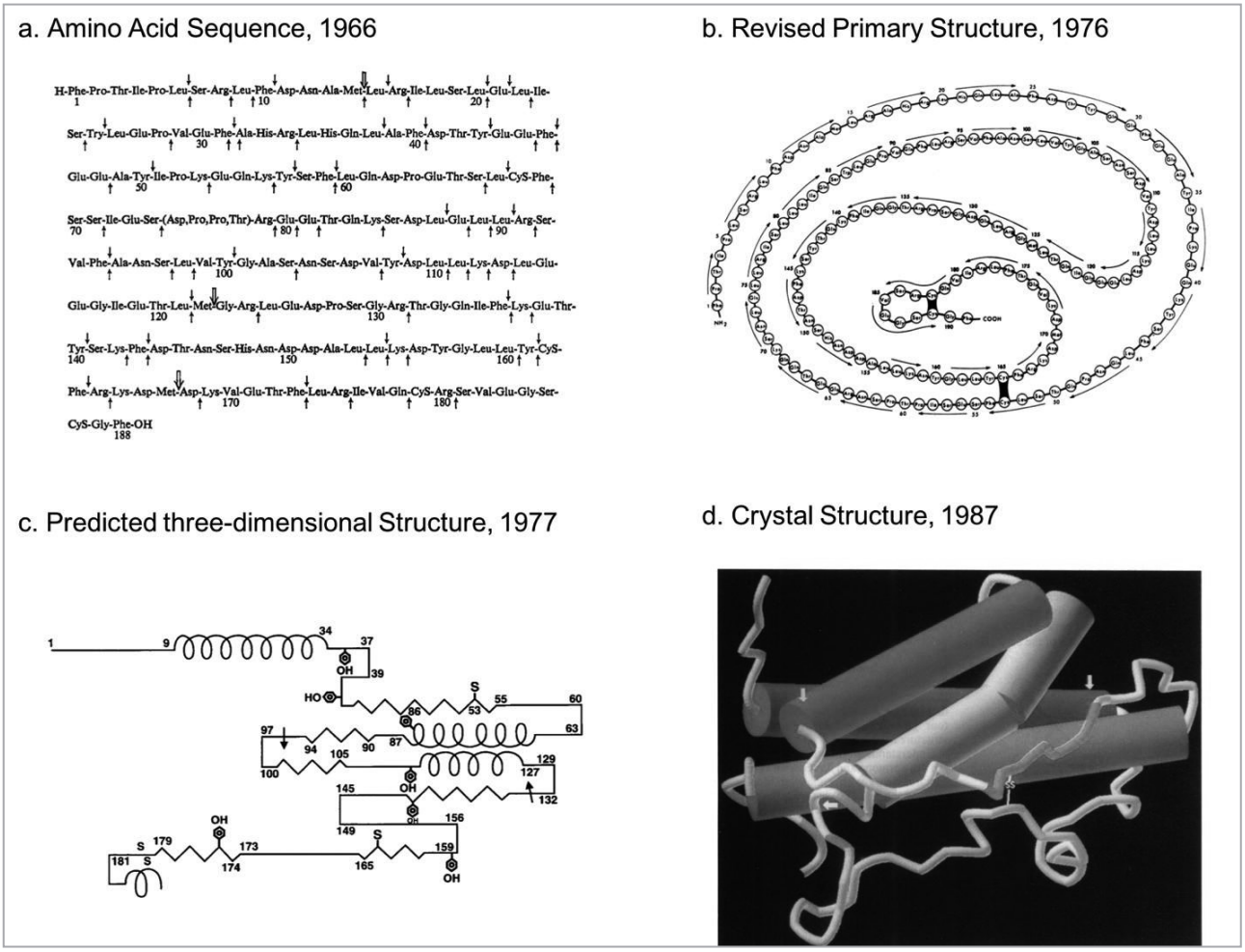

Figure 2. Evolution of growth hormone structure

a. *Amino acid sequence of hGH originally proposed by Choh Hao Li et al. Each arrow represents cleavage sites by trypsin, chymotrypsin, pepsin, and cyanogen bromide.*

b. *Primary structure of hGH revised by Li et al.*

c. *Schematic diagram of possible conformational state of bovine GH proposed by Sonenberg et al.*

d. *Crystal structure of porcine GH elucidated by Abdel-Meguid et al.*

reference (70) for an in-depth review. Despite the work of many and an almost inexhaustible number of fragments, an "active core" remains elusive to this day.

Understanding the structure of GH and its receptor are essential to understanding function. Therefore, it was ultimately exciting when Dr. Abdel-Meguid *et al*. first determined the crystal structure of porcine GH in 1987 (**figure 2d**) (71). Later in 1992, GH was co-crystalized along with two extracellular domains of its receptor by de Vos *et al*. (72). However, it was Dr. Michael Waters and his colleagues who were first to purify and characterize the GH receptor where they discovered that the GH binding protein corresponds to the extracellular domain of the receptor (73). Remarkably, in 2014, Waters along with Dr. Andrew Brooks *et al*. identified the structural changes (rotation) that occur when GH binds its receptor (**figure 3**) (74) which is now considered the dogmatic mechanism by which GH acts.

Discovery of Pegvisomant

Using evidence from many of their predecessors, Dr. John Kopchick and his graduate student, Wen Chen, discovered a GH receptor antagonist. Specifically, two fundamental pieces of evidence, the idea that different regions of GH contain specific activities and that fragment 96-133 (as presented by Sonenberg and colleagues) may be the portion of GH that promoted bone growth, led to initiation of their studies. Together with site-specific mutagenesis and the creation of transgenic mice that express these site-specific changes, they were able to determine that a growth promoting region of GH is in the third alpha helix. The third alpha helix was of interest because of its imperfect amphipathic character. In bGH, substituting Glu 117 with a hydrophobic amino acid as well as substituting Gly 119 and Ala 122 with hydrophilic amino acids would create a perfect amphipathic alpha helix and hopefully increase the activity of the molecule. When these changes were made, the molecule bound to the GHR with the same affinity as native GH, however, when expressed in transgenic mice, small mice resulted (75).

Further control experiments showed that substitution of one amino acid, Gly 119 in bGH (Gly 120 in hGH) for Lys, resulted in a classic receptor antagonist. It was found that it bound to GH receptors *in vitro* with a similar affinity as native GH. *In vivo* expression of this protein resulted in dwarf mice (76). This single amino acid change in the GH molecule became the foundation for the GH receptor antagonist, which led to the drug now known as SomavertTM (pegvisomant for injection). It is pegylated to prolong its serum half-life as well as to prevent antibody formation and is now used as a safe and efficacious compound for treating acromegaly (77,78).

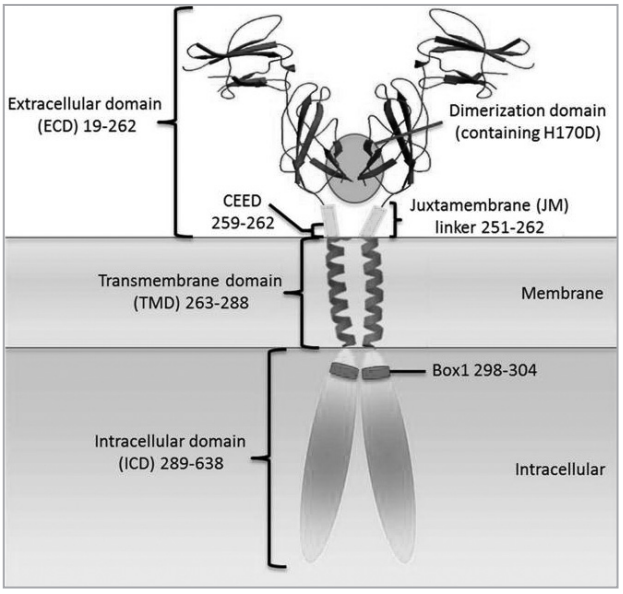

Figure 3. A figure by Waters et al. (2014) shows the significant portions of the receptor binding of GH to the dimerized GH receptor results in rotation of the receptor
Consequently, the intracellular domains move apart from one another allowing for activation of JAK2.

Conclusion

As with any scientific discipline, results are attributed to the work and passion of our predecessors. It is often said that we 'stand on the shoulders' of others. In the GH area, this statement is definitely true. The current researchers are indebted to the work of their predecessors, some of which is presented above. In this short summary, we obviously have not presented 'all' of the individuals who have contributed to the tremendous literature in the GH area. For that, we apologize. In this regard, within subsequent chapters in this volume, other important contributions will be presented. As we were writing this chapter, we became aware of an excellent review of the history of GH: 'Growth Hormone - past present and future' by Michael B. Ranke and Jan M. Wit (79). The reader is referred to this excellent review. Additionally, a time line of discoveries related to GH as depicted in the review is awesome. Thank you, Jan and Michael.

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Disclosure

MB and SB have no conflicts of interest. JK discovered GH Receptor Antagonists, one of which (Somavert; pegvisomant for injection) has been approved for use in patients with acromegaly.

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The Era of Cadaveric Pituitary Extracted Human Growth Hormone (1958 – 1985): Biological and Clinical Aspects

Zvi Laron, MD, PhD (hc)

Endocrinology and Diabetes Research Unit, Schneider Children's Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Israel

Corresponding author: Zvi Laron MD PhD (hc), Endocrinology and Diabetes Research Unit Schneider Children's Medical Center, 14 Kaplan Street, Petah Tikva 4920200, Israel, E-mail: laronz@clalit.org.il

Abstract

The first patient treated with cadaveric pituitary GH (hGH) was reported in 1958. Subsequently, collection of cadaveric pituitaries started in many countries and several centers extracted the hormone using one of two methods:

a. *Acetone preservation and extraction with hot glacial acetic acid (Rabin method)*
b. *Collection in distilled water, freezing and extraction on columns yielding several pituitary hormones including hGH (Wilhelmi method).*

The purified extracts of hGH were found to have metabolic and growth stimulating activity but the limited amounts permitted the treatment only of children with GH deficiency (GHD). The purified hormone also permitted the development of specific radioimmunoassays enabling the study of the physiological and pharmacological actions of GH.

In 1985 a number of patients treated years before with Wilhelmi hGH were diagnosed with Creutzfeld-Jacob-Disease (CJD). This led to the arrest of hGH production and the use of the then recently developed biosynthetic recombinant hGH.

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***Key words**: Human Growth Hormone, Pituitary extracted GH, Early history, Pharmacology of hGH, Physiology of hGH, Biological effects of hGH, GH stimulation tests*

Early Human Pituitary Extracted Growth Hormone (hGH)

Purified human GH (hGH) extracted from cadaveric pituitaries collected in acetone was first reported by Raben in 1957 (1). His procedure using hot glacial acetic acid retained GH but destroyed TSH, LH and FSH. Wilhelmi on the other hand trying to preserve all pituitary hormones froze the pituitaries collected in distilled water (2). The first treated patient was reported by Raben in 1958 (3). By 1962 Raben received 15,000 pituitaries per year and Wilhelmi 3,000. They were followed by many centers worldwide including ours, and National Pituitaries Agencies were established. About 400 pituitaries per year were needed to treat one child with hypopituitarism.

The first assays to establish the potency of hGH used the growth rate of rat tails, expressed in Units/mg. Next followed the rat tibia test measuring the growth of the epiphyseal cartilage (4). An assay based on the immunological properties of hGH was the hemagglutination-inhibition test (5).

The pituitaries extracted by Wilhelmi method yielded more GH by gland than those extracted by the Raben method (6).

Actual standards were developed only after 1977 by Parlow at NIH.

Radio-Immunoassay of hGH

The purification of hGH enabled its labeling with radioactive iodine (7,8) and the development of radioimmunoassays (9,10). This permitted the measurement of levels of GH in normal and abnormal states, making the diagnosis of GH deficiency (11), and GH insensitivity (12).

It was the beginning of a new era of studies of the physiology, pharmacology and biology of human Growth Hormone.

Regulation of GH Secretion

Sequential measurements of serum GH concentrations revealed that GH is secreted in pulses mainly at night (13,14) or by stress conditions regulated by the hypothalamic growth hormone releasing hormone (GHRH) and somatostatin, the GH inhibiting hormone (15). Most basal levels were low (16). These findings led to the necessity to develop GH stimulation tests to determine GH sufficiency or insufficiency as an indication for treatment with the limited amounts of available clinical grade hGH.

Growth Hormone Stimulation Tests

The observation that hypoglycemia is a potent stimulus of GH secretion (17,18) led to the intravenous (I.V.) insulin tolerance test for pituitary GH sufficiency (19,20). Further stimulation tests developed were the I.V. arginine infusion (21), intramuscular glucagon injection (22,23), the comparison of the two (24), oral levodopa (25,26), and subsequently the now frequently used oral clonidine GH stimulation test (27).

Finding that sex hormones increase the secretion of GH (28,29) led subsequently to the proposal of sex hormone priming in prepubertal children (30). Growth hormone secretion was found to be dependent on normal thyroid function (31), necessitating testing for thyroid hormone sufficiency before GH stimulation.

The simple exercise test (32,33) was abandoned due to only 50-60% reliability (34).

The Biological Half-Life of Injected hGH

The I.V. injection of 20-50 μc of ^{131}I hGH to adolescent boys and girls showed a rapid t½ phase of 20-30 min and a slow phase of 150-160 min. (35).

The I.V. administration of unlabeled and of 200μc ^{131}I labelled hGH to young adults of both genders showed a t½ of 17-30 min, whereas intramuscular injection revealed a disappearance within 95-125 min (36). The t½ disappearance of ^{131}I hGH in patients with acromegaly was slower due to the high levels of endogenous GH (37).

Other studies reported the localization of ^{131}I hGH in various tissues (38).

Acute and Short Term Effects of Pituitary Extracted hGH

Intravenous injection of hGH increased serum free fatty acids in adults (39,40) and intramuscular administration of hGH 3 times weekly to children with GH deficiency resulted in a reduction in the subscapular skinfold thickness (40). This effect of hGH was reduced in obese children (41) and adults (42). Administration of hGH also caused sodium and water retention (42), reduced blood urea, a decrease in urinary sodium, potassium and urea excretion, and an increase in calciuria (43). Ikkos et al (44) described that hGH administration in large doses caused diminished carbohydrate tolerance, hyperglycemia and glycosuria.

Long-Term Treatment with hGH

As the availability of purified cadaver pituitary extracted hGH preparations was limited, only few centers had the possibility to use such preparations. The first proof of the growth promoting effect of such a preparation was reported by Raben in 1958 (3) in a 17 year-old male with isolated GH deficiency (IGHD). In addition to the linear growth spurt, he noted a rise in serum inorganic phosphorus and alkaline phosphatase. These studies were followed by others demonstrating linear growth stimulation (45). hGH treatment stimulated growth also in very short children without GH deficiency (46), but ultimately its use was restricted only to children with proven GH deficiency. Due to the restricted quantity of hGH available, intermittent therapy was tried (47,48). The combination of hGH and an androgen in children with multiple pituitary hormone deficiencies (MPHD) further increased the growth rate (49). Using the administration of commercially produced hGH, more studies of long-term treatment followed (50-55). In the first year, the catch-up growth was greater than in the following years; however even with intermittent treatment children with GH deficiency reached near normal heights (**figure 1**). Of note is that the below-normal head circumference in untreated children with GH deficiency responded with a rapid catch-up growth (56), even faster than the linear growth (**figure 2**). Long-term hGH administration also increased penile length and testicular volume (57).

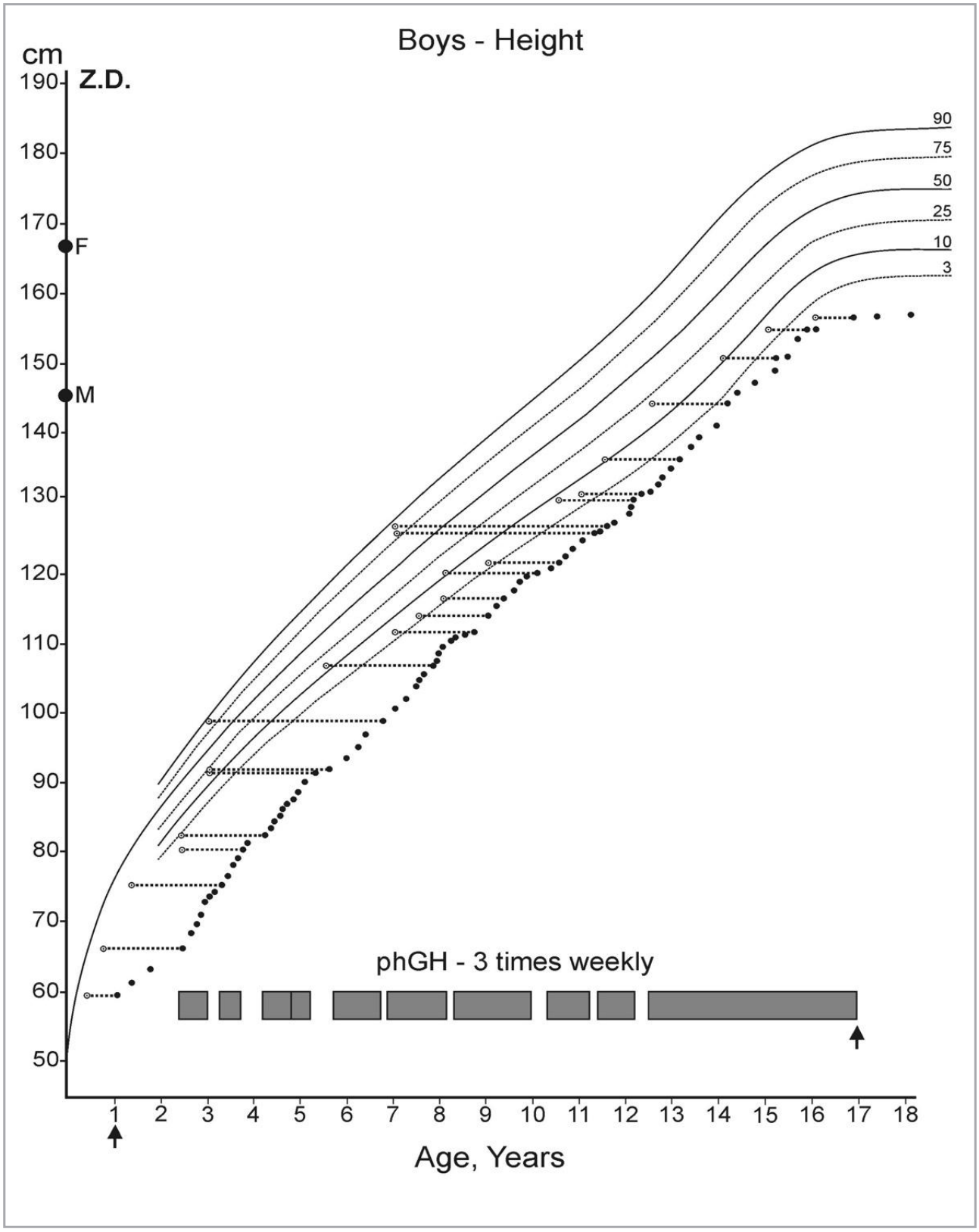

Figure 1. Longitudinal growth before and during intermittent hGH treatment of patient ZD with congenital isolated GH deficiency
***O** = bone age, **M** = mother, **F** = father*

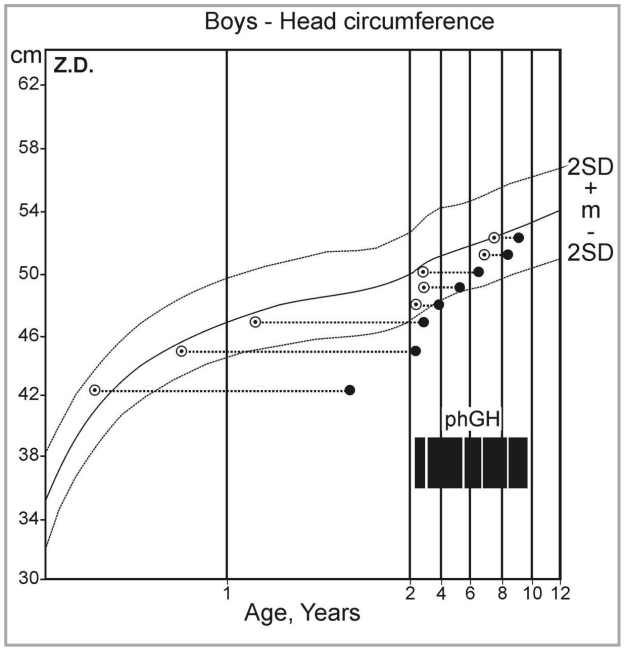

Figure 2. Head circumference growth before and during intermittent hGH treatment of patient ZD as in figure 1
***O** = bone age*

Adverse Effects

Zuppinger and Joss (58) and Costin *et al* (59) described that administration of hGH, to children with GHD caused impaired carbohydrate metabolism.

The use of several preparations of hGH, mainly the earlier ones, caused development of anti-hGH antibodies (60), some of them blocking antibodies (61,62).

The most serious adverse effect of hGH was Creutzfeld-Jacob Disease described below.

Creutzfeld-Jacob Disease (CJD)

Creutzfeld-Jacob Disease is a transmissible spongiform encephalopathy caused by prions (63). It includes the sheep disease scrapie (64) and bovine spongiform encephalopathy (BSE). It occurs sporadically in many countries (65) and known clusters, often familial, as reported from Slovakia (66), Hungary (67) and among Lybian Jews (68). Person to person transmission and Iatrogenic transmission of CJD by a corneal graft from a donor who had died of undiagnosed CJD was reported by Duffy *et al* (69). Transmission of CJD also has been shown following neurosurgery, stereotactic electroencephalography, and dura mater implants (70). The incubation period and clinical phenotype of the ensuing illnesses vary with the route of inoculation, usually 15-20 years with a predominantly cerebellar onset.

In 1985 CJD was diagnosed in 3 North American patients treated with hGH prepared by the U.S. National Pituitary Agency before 1977 (71), followed by further reports from the pool of about 7000 patients who had been treated with hGH distributed by this agency (72). One batch of hGH produced between 1966 and 1969 by the Wilhelmi method was used in every one of these patients (73).

Following the announcement in the USA, surveillance studies in other countries followed (74) and patients were diagnosed up to 2006 and beyond (75), of these 194 were related to hGH (**table**). Affected patients also were reported from the UK (76), New Zealand (77), and Brazil (78). Striking is the high number of patients in France (75,79).

Table. Reported number of patients with Creutzfeld-Jacob Disease related to pituitary extracted hGH

Country	Number of patients
Australia	1
Brazil	1
Holland	1
Quatar	1
New Zealand	6
USA	26
United Kingdom	51
France	107
Total	194

Modified from Brown et al (75)

It became evident that CJD prions were transmitted by frozen pituitaries from affected patients and extracted by the Wilhelmi method, whereas hGH preparations using collection of pituitaries in acetone and extracted by the Raben method (1) with hot glacial acetic acid with or without the addition of 6M urea, did not cause CJD (80-82).

The development of unlimited quantities of biosynthetic recombinant human GH (rhGH) in that period (1984-85) (83,84) led the USA in 1985 and followed by all other countries, to stop the production of pituitary extracted hGH.

Disclosure

The author has nothing to declare.

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Gene Sequence and Production of Recombinant MetGH/hGH

John S. Parks, MD, PhD

Emeritus Professor of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322, USA

Corresponding author: John S. Parks, MD, PhD, 209 Glendale Avenue, Decatur, Georgia 30030, USA, Tel: 1-404-556-0522, E-mail: jsparks4410@gmail.com

Abstract

Recombinant human growth hormones were the products of a revolution in biotechnology that took place in the San Francisco Bay area of California in the 1970's. A combination of Herb Boyer's restriction enzymes with Stanley Cohen's bacterial plasmids provided the power to select and amplify virtually any gene. The complementary personalities and talents of Herb Boyer and Robert Swanson led to formation of Genentech and with it the development of a product that overcame the limitations of scarcity and the risks of slow virus contamination inherent in extracted pituitary growth hormone. The extra amino acid in metGH was dropped and other manufacturers joined in the effort to explore indications for rhGH beyond the replacement of a missing hormone. After more than thirty years of availability and careful study, we still have much to learn about the safe and effective use of rhGH.

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Background

The 1970's were an exceptional decade for molecular genetics. At the beginning of the decade gene modified lambda bacteriophage were used to select and overexpress genes indigenous to Escherichia coli (1). There was no way to persuade bacteria to express animal proteins. Over the next few years, great breakthroughs were made in the understanding of plasmids, restriction endonucleases and growth hormone gene structure. By the end of the decade, these elements had been combined with entrepreneurial determination to achieve the goal of synthesis of recombinant human growth hormone.

The Birth of Recombinant DNA Technology

The path to rhGH was forged in the San Francisco Bay area, by principals at the University of California, San Francisco (UCSF) and Stanford University. They were, from left to right in **figure 1**, Stanley N. Cohen, MD, Herbert W Boyer, PHD, and Robert Swanson, who had a bachelor's degree in chemistry and master's degree in from the Massachusetts Institute of Technology. There is a wealth of information about their careers. Along with thirty other leaders in bay area biotechnology, they participated in an oral history project conducted through the Bancroft Library of the University of California, Berkley. Most of these interviews are available on line (2). Sally Smith Hughes, the lead interviewer, drew upon them in writing Genentech: The Beginnings of Biotech, published in 2011 (3). It provides a detailed analysis of personalities, goals and discoveries contributing to development of a revolutionary new technology and the development of rhGH.

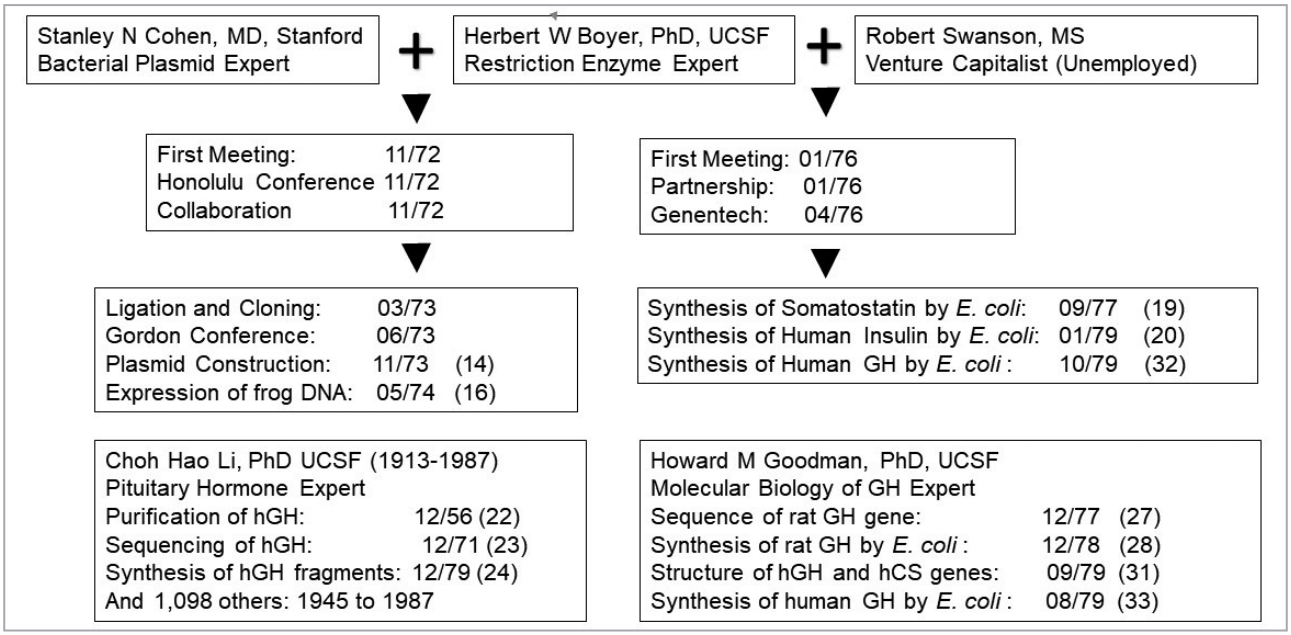

Figure 1. Principals, Areas of Expertise, Meetings and Accomplishments in the Race for Recombinant Growth Hormone
This chart illustrates the connections among individuals involved in the development of metGH and rhGH during the 1970's. The dates of meetings are indicated by month and year. Major achievements are indicated by the month and year of publication together with the reference number in this paper's reference list.

Dr. Cohen began studying plasmids during a post-doctoral fellowship at Albert Einstein School of Medicine in New York. He continued this work, despite a heavy clinical load, after being appointed Assistant Professor of Medicine in the Division of Hematology at Stanford in 1968. Plasmids are small DNA molecules that replicate independently of the host cell's DNA. Some, termed R-factors, carry genes that confer antibiotic resistance. In 1972, Dr. Cohen found that treatment with calcium chloride enabled Escherichia coli to take up isolated R-factor DNA and produce progeny containing functional plasmids (4). About one in a million cells was transformed, but the transformed cells could be selected, cloned and maintained because of their antibiotic resistance. Plasmid DNA needed to be broken into smaller pieces to analyze the functions and structures of individual genes. Sonication produced DNA fragments, but the breaks were random (5). There was a pressing need for a means of generating defined and reproducible fragments. Dr. Cohen was organizing a conference on plasmids in Honolulu, Hawaii, to be held in November 1972. A colleague had heard of Herb Boyer's work with the bacterial restriction enzymes and suggested to Dr. Cohen that he should invite Dr. Boyer (6).

Herb Boyer came to UCSF in 1965 as an Assistant Professor of Microbiology after PhD training at the University of Pittsburgh and post-doctoral training at Yale University. His work centered on bacterial restriction enzymes (7). These enzymes had evolved to protect bacteria against infection by plasmids, bacteriophage and other invaders. They recognize and cut within specific DNA sequences. Recognition sites are generally 4 to 8 bases in length and are palindromic with a central axis of symmetry. One example is Eco RI, the first such enzyme to be isolated from Escherichia coli (8). The sequence 5'GAATTC3' on one strand is mirrored by 3'CTTAAG5' on the complementary strand. The enzyme cuts between G and A on each strand and generates staggered, sticky ends which anneal to complementary sequences, much like the components of mortise and tenon joints used in carpentry (9). Following this analogy, the matched DNA fragments can then be glued together with DNA ligase. Dr. Boyer brought his new information about Eco RI to the plasmid meeting in Honolulu.

Dr. Cohen saw the relevance of Eco RI to his work with plasmids. He and Dr. Boyer had a long conversation over sandwiches and beer (3). They decided to collaborate and began work on the new project in January 1973. The Cohen lab worked on finding a suitable plasmid and on doing transfection experiments. The Boyer lab handled the enzymology, with Bob Helling doing electrophoresis and characterization of DNA fragments. Annie Chang, who lived in San Francisco ferried specimens the 35 miles between the two laboratories (3). The work went very rapidly, with positive results in March and publication in September 1973.

Dr. Cohen's team searched for a plasmid with a single Eco RI site that could be opened to convert the circular plasmid DNA in a single strand with cohesive ends. They found a small plasmid, called pSC101 (for plasmid Stanley Cohen

number 101), which carried a tetracycline resistance gene and had a single Eco RI site. When a digest of pSC101 DNA was mixed with a digest of DNA from another plasmid, called R6-5, carrying the kanamycin resistance gene and used to transfect Escherichia coli, some of the bacteria grew in the presence of both tetracycline and kanamycin. Electrophoresis and ethidium bromide staining of plasmid DNA extracted from the cloned colonies indicated a recombinant DNA molecule. It contained all of pSC101 and a short segment from R6-5 that coded for kanamycin resistance. Sally Hughes describes the moment this way, "The sight brought tears to Boyer's eyes: here before him was evidence of a simple method for isolating and accurately copying specific genes and DNA fragments in virtually endless quantity" (3).

Despite Cohen's desire to keep their findings secret until formal publication, Boyer couldn't wait to share the good news about their revolutionary finding. He presented the work informally at the June 11 to 15, 1973, Gordon Research Conference on Nucleic Acids (3). Some attendees saw this as a breakthrough that would have an impact on the directions of their own research.

Others considered it as an opening of Pandora's box. The next morning the conference chair, Maxine Singer, noted the "fascination with an evolving understanding of these amazing molecules which may lead to useful alleviation of human health problems" but went on to emphasize "potential hazards such molecules may engender" (10). She limited further discussion and asked for a show of hands to vote on a proposal to send a letter from the conference to the Presidents of the National Academies of Science and Medicine. The ayes prevailed with a majority of 78 out of about 95. Her letter to Dr. Phillip Handler requested formation of a study committee to consider the problem and recommend specific actions or guidelines. Paul Berg was appointed to be the chair of the resulting committee. In July 1974 the Berg committee published a letter co-signed by Boyer and Cohen and eight other prominent scientists which proposed a voluntary moratorium on creating new plasmids carrying DNA antibiotic resistance or bacterial toxins and experiments linking DNA from linking animal viruses to plasmids or viral DNA (11). The committee also recommended an international meeting of involved scientists to review scientific progress and further discuss ways to deal with potential hazards of recombinant DNA. This meeting at the Asilomar conference center in February 1975, led to the formulation of guidelines that were adopted to varying degrees in different countries to assure biological containment and address some of the public objections to implications of the new technology (12,13).

The landmark paper on recombinant DNA was published in the Proceedings of the National Academy of Science in November, 1973 (14). Additional papers within the following year showed that the Cohen and Boyer procedure worked for cloning recombinant molecules from plasmid and staphylococcal DNA (15) and from plasmid and frog DNA (16). It appeared that there were no limits to the ability splice and clone genes from any species. Stanford's Office of Technology Licensing saw an opportunity to patent the process on behalf of Stanford and the University of California and did so on November 4, 1974, just short of one year after the original publication (17).

The Origins of Genentech

Robert A Swanson was 26 years old in 1975. He had graduated from Massachusetts Institute of Technology with bachelor's degree in chemistry and a master's in business and was working as a junior partner in the Kleiner and Perkins venture capital investment firm. His assignment was to monitor the partnership's investment in Cetus. Donald Glaser, one of three founders of Cetus was enthusiastic about the emerging recombinant DNA technology, but the other two were not (18). Swanson tried to convince them to set up a division to exploit the opportunities it presented, but to no avail. Like others in the industry, they were waiting for someone else to show that it could be used to produce a marketable product.

Bob Swanson was fired from Kleiner and Perkins at the end of 1975 and began to collect unemployment checks. With free time available, he made phone calls to scientists who had participated in the Asilomar DNA conference. The ones he reached agreed the technology had promise, but felt that it would require "a decade or two" before it could become profitable. Swanson eventually reached Herb Boyer and told him he wanted to start a company. Boyer was characteristically friendly, but very busy. He agreed to give Swanson 10 minutes of his time that Friday afternoon, January 17th 1976. (18). The meeting started well and they adjourned to a local tavern to continue their three hours conversation. This event is now memorialized in a life-size bronze statue by sculptor Larry Anderson located outside a research building on the Genentech campus. (18). Swanson learned that Boyer was not only a practitioner, but one of the developers of gene splicing. Boyer mentioned that he had some experience with growth hormone. His oldest son, who was short for age, had been tested by a pediatric endocrinologist and had been found not to have GH deficiency. At the time, Boyer had mentioned to his wife, Gracie, "You know, we could make hGH. All we have to do is isolate the gene". Swanson, in turn, convinced Boyer that he had the business and finance expertise to start a company. Swanson recalled "I can't be sure whether it was my persuasiveness, Herb's enthusiasm, or the effect of the beers, but we agreed that night to establish a legal partnership to investigate the commercial feasibility of recombinant DNA technology" (18). Each agreed to ante up $500 to apply to legal fees. The envisioned company's name was to be Genentech, which is an abbreviation of Genetic Engineering Technology.

The path to developing, producing and marketing recombinant human GH required a number of preliminary steps. Genentech did not have any laboratory space or money. GH was a moderately large hormone containing 191 amino acids. The business plan dictated contracting with an established pharmaceutical firm to produce a smaller hormone. Swanson and Boyer chose insulin. It was considerably smaller, with 51 amino acids. There was huge market for it and a reasonable expectation that recombinant human insulin could be produced at a lower cost than pork or beef insulin. There was great skepticism that this goal could be achieved (18).

In early 1976, Boyer contacted Arthur Riggs and Keiichi Itakura at the City of Hope National Medical Center in Duarte, California about the idea of collaborating on the insulin project. They convinced Boyer to start with synthesis of somatostatin which was a 14 amino acid hormone. A chemically synthesized coding sequence was fused to DNA from a plasmid containing the ß-galactosidase gene. Clones of E. coli transformed with chimeric plasmid DNA contained a fusion protein from which somatostatin could be released by cleavage with cyanogen bromide (19). This represented the first example of synthesis of a functional polypeptide product from a chemically synthesized gene.

With the proof of concept established, they were able to receive additional financial support. The team expanded, moved to a small warehouse lab and shifted its focus to human insulin. They saw themselves in competition with Walter Gilbert's lab at Harvard and William Rutter and Howard Goodman's at UCSF. The other labs were working with cDNA and were hindered by recombinant DNA guidelines which applied to animal and viral sources of DNA but not to synthetic DNA. The Genentech and City of Hope researchers produced separate oligonucleotides for the insulin A chain and B chains, fused them separately to the ß-galactosidase gene, transfected E coli and expressed the fusion proteins. The insulin chains were cleaved from the fusion proteins, purified, mixed, reduced and re-oxidized to generate intact human insulin. This landmark was achieved on August 21, 1978 (18). Within four days, Eli Lilly signed a contract with Genentech and the City of Hope which gave Lilly the exclusive worldwide rights to manufacture and market human insulin using Genentech's technology. The paper describing recombinant insulin production was published in January 1979 (20).

The Race to Make Recombinant Human Growth Hormone

Human growth hormone was the next target for Boyer and Swanson. They had signed a research and development contract with Kabi Vitrum, the leading commercial producer of extracted pituitary GH, in August 1978 (21). Dr. Boyer had very little prior experience with the hormone, compared to other scientists at the University of California, San Francisco. Others at UCSF had infinitely more.

Choh Hao Li, PhD directed the Hormone Research Institute and was the grand old man of pituitary hormone chemistry. He had published more than 1,000 articles on the isolation, structure and function of pituitary hormones over the preceding four decades. Achievements included isolation, sequencing of and partial chemical synthesis of hGH. (22-24) Selna Kaplan and Melvin Grumbach in pediatric endocrinology at UCSF had discovered growth hormone's close relative, human placental lactogen or human chorionic somatomammotropin (25) as well as being leaders in clinical studies of growth hormone (26).

Howard Goodman, PhD, and his research group in the Department of Biochemistry and Biophysics had a stellar record of growth hormone research, relating to the lipolytic activity, regulation and molecular biology of GH. His laboratory group had scored a number of academic breakthroughs in expanding scientific understanding of genes and hormones related to hGH. They sequenced the rat GH gene in 1976 (27) and achieved synthesis of rat GH by bacteria in 1978 (28). They were also exploring evolutionary relationships among prolactin genes (29) and the cluster of human genes encoding human growth hormone and chorionic somatomammotropin (30,31). Theirs was a broad focus on pursuit of scientific knowledge and publication, much more than on commercial gain. Nevertheless, they had signed a contract with Eli Lilly to work on recombinant expression of human insulin and human growth hormone.

Genentech's primary focus was making a product to be able to grow the company, become a full-fledged pharmaceutical company and eventually yield a profit. Dr. Boyer knew where to find young and eager scientists with experience and productivity. He recruited David Goeddel and Dennis Kleid from Stanford, Roberto Crea from the City of Hope and Peter Seeburg and Axel Ullrich from Dr. Goodman's lab (21). As a post-doctoral fellow, Seeburg had made major contributions to the projects on rat GH and had been encouraged by John D. Baxter, MD, to work on human GH expression. Reportedly, he was disappointed that he had not been included on a UCSF patent application based in part upon his work. He brought some of his biological samples with him before leaving UCSF on October 31, 1978, then returned with Axel Ullrich, PhD. on New Year's Eve, 1978, to pick up some more (21). Drs. Goodman and Baxter wrote to tell him to return all his samples, but Dr. Seeburg did not comply.

The work products of the two competing teams are compared in **table 1** and the processes are described in the next two paragraphs.

The Genentech investigators chemically synthesized a DNA "adaptor" fragment with an ATG translation initiation codon preceding the coding sequence for amino acid residues 1 to 23 of hGH. This DNA was combined with restriction fragments of plasmid and the desired clones were identified. A second recombinant plasmid was constructed using a restriction fragment of hGH cDNA containing the codons for amino acids 24 to 191. The two separately cloned hGH DNA fragments were treated with restriction enzymes and ligated *in vitro*. A fragment containing the desired 591 base pair product was purified and inserted into an expression plasmid containing the E. coli lac promoter. E. coli containing pHGH107-1 produced up to 2.4 mcg of immunoassayable hGH or nearly 200,000 copies per cell growing in log phase in rich media. Auto-radiography of labeled product was consistent with a 192 amino acid protein with an extra methionine at the amino terminus (32).

Joseph Martial and colleagues at UCSF isolated mRNA from human pituitary tumors and cloned cDNA representing the 29 nucleotides in the 5' non-coding region, the coding regions for the 26 amino acid signal peptide and the 191 amino acid mature hormone, together with 108 nucleotides of the 3' untranslated region (33). This cDNA was ligated, in the correct reading frame, to the E. coli Tryptophan D gene in a plasmid vehicle and used to transfect E. coli and clones were made from ampicillin resistant colonies. Two proteins increased markedly after exposure of cells to 3b-indoylacrylic acid, a de-repressor of the E. coli trp operon. One was the trpE gene product and the other was a 32,000-dalton fused trpD-hGH product that could be immunoprecipitated by anti-hGH antibodies and constituted about 3 percent of total bacterial protein synthesis.

The UCSF researchers did not describe a way to remove and recover hGH from the fusion protein. When interviewed, John Baxter said that the difficult research had been accomplished and that removing the E coli and signal peptide sequence would involve engineering and not additional research (34).

Martial's UCSF manuscript was submitted on June 8 and published on August 10, 1979 (33). Goeddel's Genentech manuscript was submitted on July 6 and published on October 18 (32). It contained thanked John Shine of UCSF for "help in planning the project" stated that "RNA used was prepared by P.H.S. while a postdoctoral fellow" at UCSF. The dispute over how much this RNA contributed to Genentech's success was settled out of court two decades later (35).

The two groups had taken different paths and arrived at two different prototypes for recombinant hGH. Goeddel's 192 amino acid metGH became Genentech's Protropin and Kabi's Somatonorm. Martial's hGH fusion protein led to Lilly's "natural sequence" 191 amino acid Humatrope brand of rhGH. Project director Linda Fryklund at Kabi had the advantage of extensive experience with their Crescormon brand of extracted pituitary hGH. Lilly did not have experience with GH, but it had a one year head start on recombinant DNA derived hormones through its work on "natural sequence" human insulin using the recombinant DNA developed by Genentech.

Table 1. Comparison of metGH and rhGH

Feature	metGH	rhGH
Company	Genentech, Kabi	Eli Lilly
Brand Names	Protropin, Somatonorm	Humatrope
Nonproprietary Name	Somatrem	Somatropin
Protein Product	metGH	trpD-hGH Fusion Protein
Molecular Weight	22,000	32,000
Maximum Expression	186,000 Copies per Cell	3% of total
Expression Plasmid	E. coli pHGH107-1	Not named in publication
Promoter	E. coli lac + lac	E. coli trp
5' Fragment	Synthetic ATG + codons 1 to 24 of hGH	E. coli trp D gene
3' Fragment	cDNA with codons 24 to 191, stop codon, dC tail	cDNA for 5' untranslated + 26 aa signal and all of GH
Ligation Site	Hae III in codon 24	Hind III in trp D and cDNA
Reference	Goeddel *et al* (32)	Martial *et al* (33)

From Plasmid to Product

In the original Genentech/Kabi production system, the metGH product remained inside the E. coli cells. Large batch cultures were grown at Genentech. Harvested cell paste was shared between Genentech and Kabi. After cell lysis, about half of the met-GH was soluble and half was not. Genentech went after both, but Kabi processed only the soluble fraction (36). The product, purified by a number of liquid chromatographic steps to greater than 99% purity, had the following properties as compared to pituitary hGH:

1. The expected sequence according to tryptic analysis and Edman degradation
2. The correct two disulfide bridges between the four cysteines
3. The same isoelectric point, but with fewer minor isohormone components
4. The correct folding and three-dimensional structure by circular dichroism
5. No unique epitopes in hyperimmunized rabbits
6. Equal potency in rat weight gain and rat tibia length models
7. Equal receptor-binding activity and down regulation activity in IM-9, transformed human lymphocytes
8. Equal binding to "lactogenic" receptors on pregnant rat liver membranes (37-41).

MetGH produced by Genentech and by Kabi was ready to begin Phase 1 tests in humans. Administration of the Genentech hormone to normal adults provoked fever and chills, despite negative results on conventional E. coli pyrogen testing. Further purification eliminated the problem. Administration of Kabi Somatonorm to 7 adult volunteers produced no acute side effects (38).

Ray Hintz and associates compared the efficacy of methGH to that of human pituitary hormone from Serono in a short-term, double-blind, crossover trial (42). Twenty-two adult male volunteers received a supraphysiological average dose of 8 mg per day for 4 days. The subjects showed mean GH peaks of about 220 ng/ml at four hours after injection, with a similar half-life of disappearance of GH. Levels of somatomedin, now termed IGF-I, tripled over 4 days, whether measured by immunoassay or by receptor-binding assay.

Clinical trials of long-term efficacy Genentech's Protropin metGH in U.S, children with GH deficiency began in September 1981. The results of 12 to 24 months of treatment were presented at a conference in 1983 (43) and the results of 24 to 40 months of treatment were published in March 1986 (44). Thirty-six children received methGH including 22 (group A) who were started on a less-purified preparation and later switched to a preparation containing a lower concentration of contaminating E. coli protein. Another 10 (group B) received the more purified methGH Intramuscular doses of 0.1 mg/kg were given three times a week (weekly dose 0.3 mg/kg/wk). Ten children (group C) received an identical dose of Crescormon. Median age at starting treatment was 9 years in the metGH groups, but the pituitary hGH group was younger with a median age of 6.6 years. Median height SDS averaged -3.7 in the three groups. Pre-treatment height velocities tripled to greater than 10 cm/yr in all three groups during the first treatment year and declined to around 7 cm/yr during the second year. Height velocity was sustained at greater than 7 cm/yr for the third treatment year in group A. Twenty-one of the 22 children in group A developed antibodies to GH, compared to 6 of 14 in group B and none of the 10 who received pituitary hGH.

Phase 3 studies of Kabi's met-hGH were done in Germany with 31 patients (45), France with 21 (46), Spain with 21 (47), the United Kingdom with 17 patients (48), and Japan with 11 (49). The results were quite similar to Genentech's pre-approval studies. The metGH was as good as extracted hGH in accelerating growth and raising somatomedin levels. There were no worrisome side effects.

Antibodies to hGH developed more frequently in children receiving earlier and less highly purified preparations, and growth responses generally did not correlate with titers of antibodies to either growth hormone or E. coli proteins. The conclusion was that antibodies were related to contaminants and that the extra methionine had nothing to do with it.

There were at least two later reports of marked slowing of growth associated with high titers of anti-GH antibodies and binding capacities above 2.0mg/L while on metGH (50,51). Both patients experienced a marked increase in growth rate when they were changed over to treatment with natural sequence 191 amino acid growth hormone.

Studies comparing metGH to pituitary hGH were terminated abruptly in April 1985, when the first cases of Creutzfelt-Jakob disease (CJD) were associated with extracted hGH. The US Food and Drug Administration asked Kabi, Serono and Novo Nordisk to withdraw their drugs from the market. Mortimer Lippsett, MD, Director of the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases (NIDDK) at NIH told doctors in the US to cease using hGH distributed by the National Hormone and Pituitary Program (52). Genentech was given temporary permission to provide met-hGH as an investigational new drug to growth hormone deficient children who required it to prevent life-threatening hypoglycemia (53).

Given the fact that Kabi could not prove that Crescormon was incapable of causing CJD, the company stopped distribution and recalled supplies from its global subsidiaries. Serono

continued distributing their product in countries where it had not been banned.

FDA approval of Genentech's Protropin was granted for treatment of growth hormone deficiency in children in October 1985 (54). Approval included designation as an "Orphan Drug" and with it seven years of marketing exclusivity and generous tax credits. The FDA also demanded that Genentech design a "market survey system" to provide safety and efficacy information about each patient who received the drug. This stipulation set the stage for the National Collaborative Growth Study (NCGS) and, in time, the Kabi International Growth Study (KIGS), Lilly's Genetics and Neuroendocrinology of Short Stature International Study (GeNeSIS) and similar projects from other companies. Kabi received Swedish approval for Somatonorm in October 1985, the same month as Genentech's approval in the United States (56). Registration in countries other than the United States followed within the year.

Lilly received FDA approval for Humatrope in March 1986 (55). The terms were similar to those for Protropin. It was also considered an "Orphan Drug" and was given a seven year monopoly on the basis of the single amino acid difference between its natural sequence rhGH and Genentech's metGH. The two companies were protected from outside competition in the United States until 1992 and 1993.

The prediction of a limitless supply of inexpensive human growth hormone was partly fulfilled. Supply could expand to fit any reasonable need, but the human hormone was not cheap. Price at introduction was about $34 per mg, only $6 less than Kabi's extracted hGH had been (56). The equivalent amount in 2018 dollars is $77. One year of treatment at a fairly low dose of 0.2 mg/kg/week would cost about $3,500 for a 10 kg patient and $17,500 for a 50 kg patient. Those were staggering amounts in the 1980's, before the development of other medical products of recombinant DNA and monoclonal antibody technologies.

Promises and Perils of Recombinant Human Growth Hormone

A conference on the "Uses and Possible Abuses of Biosynthetic Human Growth Hormone" was been held in Baltimore, Maryland on November 22 and 23, 1983, before CJD and before FDA approval of recombinant GH. Dr. Lipsett invited nearly 50 pediatric endocrinologists, psychologists, ethicists and representatives from NIH, the FDA and industry to consider issues that would be raised by the availability of unlimited supplies of rhGH. Louis Underwood reported on the conference in the August 30, 1984, issue of the New England Journal of Medicine (57). Three working groups addressed biomedical aspects of growth hormone therapy, psychological and ethical issues, and regulation. There was agreement that "enormous pressure would be put on physicians to prescribe GH", no agreement on the best way to diagnose GH deficiency, agreement that the hypotheses of "bioinactive GH" (58) and "normal variant short stature" (59) had not been proven, and agreement that children with more common forms of short stature should only be treated in approved protocols. Much more needed to be learned of the impact on quality of life. Regulation and safety issues included long as well as short term health impact and how to keep GH out of the hands of athletes. There is still a lack of consensus on these issues some 35 years later.

An International Workshop on Advances in Research on Human Growth was held from September 14 to 16, 1986, at the Airlie House conference center in a rural setting outside of Washington, DC. The mood a year after approval of Protropin, Humatrope and Somatonorm was very different. Gina Bari Kolata, a science writer for Science Magazine and later for the New York Times, covered on the event (60). She reported that participants felt it was a "sure bet that the companies are not just planning to market the drug for treatment of pituitary dwarfs." During interviews held in a convivial basement bar, Barry Sherman, MD, then Director of Medical Research at Genentech said, "Obviously, there aren't that many short kids to treat." I was said to be worried about the ethics of what I called "cosmetic endocrinology". That term remains applicable today in its original form or as endo-cosmetology (61,62). James Tanner commented that GH treatment may become as common as orthodontia. He also told Mrs. Kolata, "We are now moving from an era in which there were too many patients chasing too little hormone, to an era in which there will be too much hormone chasing too few patients." Robert Blizzard, MD, weighed in on the potential benefits of rhGH in combating obesity and aging. Altogether, it sounded like physicians might be embarking on a binge of prescribing GH.

A Proliferation of Products

Table 2 lists marketed growth hormone preparations, their producers, starting dates and ending dates and approved indications. It represents a modification and updating of **table 1** in Franklin and Geffner's 2008 review (63). Genentech's U.S. 7 year monopoly on metGH expired in 1992 and Lilly's U.S. monopoly on rhGH expired in 1993. Nobody wanted to make metGH anymore, but Genentech certainly wanted to make rhGH. They and Kabi had solved the dilemma of getting rid of the amino-terminal methionine a decade before, using a new signal sequence to direct the protein through the first of two plasma membranes into the periplasmic space of E. coli (64). The result was a correctly folded, soluble natural sequence rhGH that could be released into buffer by lysis of the outer plasma membrane. Genentech received approval for Nutropin in 1992. The 10 year marketing agreement between Genentech

and Kabi expired and Kabi-Pharmacia received FDA approval to market Genotropin in the US in 1995 (65).

Serono received approval of Saizen in 1996. Unlike the other products which are produced in E. coli, Saizen in expressed in C127 cells, a mouse mammary cell tumor line transfected with polyoma virus DNA. The rhGH product is directly secreted into the culture medium. Serono had the interesting strategy of gaining separate registration for two additional products with special branding for special applications; Serostim in 1996 for AIDS wasting and Sorbtive in 2004 for short term treatment of children with short bowel syndrome. The last new rhGH product to enter the US market in the 1990's was Norditropin, produced by Novo Nordisk.

Two biosimilar rhGH products have approved in the United States. Tev-tropin received approval in 2005 and Omnitrope was approved the next year. This "biosimilar" category in the U.S. and Europe was a pathway for additional companies to produce rhGH and other products after marketing protection for the reference biological product expired (66,67). Approval requires review of the manufacturing process, demonstration of comparability to a reference product with respect to structure, purity, toxicity and effectiveness in a smaller number of patients than is ordinarily required for phase 3 studies. Efficacy for one indication, such as childhood growth hormone deficiency, is taken to imply efficacy for all approved indications.

Of the 6 currently marketed rhGH preparations listed in **table 2**, all are approved for treatment of childhood growth hormone deficiency and for adult growth hormone deficiency. Three were approved for Turner Syndrome, with the earliest approval in 1996, small for gestational age short stature (2001), and idiopathic short stature (2003). Nutropin is approved for chronic renal insufficiency pre-transplant (1993), Genotropin for Prader Willi syndrome (2000), Humatrope for SHOX haploinsufficiency (2006) and Norditropin for Noonan syndrome (2007). The United States approval dates for specific indications are generally concordant with those for Europe and Japan (68,69). Approval for a particular indication in the U.S. does not imply that the drug has been approved for that indication in other markets.

Table2. Human Growth Hormone Products, Producers, Dates and Indications

Product	Producer	Begun in US	Ended in US	Eventual Indications
Extracted Pituitary hGH	(Somatropin)			
Asellacrin	Serono	1976	1986	CGHD
Crescormon	Kabi	1970[1]	1985	CGHD
Nanormon	Novo-Nordisk	1973[1]	1985	CGHD
Recombinant 192 Amino acid Met-GH	(Somatrem)			
Protropin	Genentech	1985	1993	CGHD
Somatonorm	Kabi	1985[2]	1987	CGHD
Recombinant 191 amino acid hGH	(Somatropin)			
Humatrope	Eli Lilly	1986		CGHD, AGHD, TS, ISS, SHOX, SGA
Nutropin	Genentech	1993		CGHD,AGHD,CRI, ISS
Genotropin	Kabi-Pfizer[3]	1995[4]		CGHD, AGHD, PWS, SGA, ISS, TS
Saizen	Serono	1996		CGHD, AGHD
Serostim	Serono	1996		Aids Wasting
Norditropin	Novo Nordisk	1997		CGHD, AGHD, NS,TS, SGA
Zorbtive	Serono	2003		Short Bowel Syndrome
Tev-Tropin	Teva[5]	2005	2013	CGHD
Zomacton	Ferring[5]	2015		CGHD
Omnitrope	Sandoz	2006		CGHD, AGHD
Long-Acting 191 Amino acid hGH				
Nutropin Depot	Genentech	1999	2004	CGHD

Abbreviations: CGHD, Childhood Growth Hormone Deficiency; AGHD, Adult Growth Hormone Deficiency; TS, Turner Syndrome; ISS, Idiopathic Short Stature; CRI, Chronic Renal Insufficiency; NS, Noonan Syndrome; SGA, Small for Gestational Age; SHOX, abnormality of the Footnotes:
[1]These dates indicate initial approval by the home country of the producers, not by the US FDA
[2]Somatonorm was approved in 55 countries worldwide, but by agreement with Genentech, it was not marketed in the US or Canada.
[3]Kabi became Kabi-Pharmacia, then Pharmacia-Upjohn and, most recently, Pfizer.
[4]Genotropin was approved in Sweeden in 1987.
[5]Rights to Tev-Tropin were acquired by Ferring in 2013 and the product was rebranded as Zomacton.

In the early days of rhGH treatment, prescribing physicians tried to match specific indications with specific products. In recent years the third-party payer together with the mail-order pharmacy decides on the choice of brands. Contracting for a favorable price has largely replaced provider or family preferences or linking of products to particular approved indications.

All of the current rhGH products represent the same 191 amino acid molecule. Quality and purity of brands and batches of rhGH are assessed by structural analysis and not by bioassays (70). Competition between manufacturers has narrowed down to innovative devices for injecting the hormone. Adherence to a regimen of daily subcutaneous injections is tedious and uncomfortable. Approximately a quarter of patients miss more than 2 injections per week (71). Rohrer, Horikawa and Kappelgaard have published an excellent review of current modes of delivery, addressing simplicity of preparation, precision, ease of injection and lessening of pain as ways to improve adherence (72).

It is important to note that none of rhGH brands are approved for slowing of the aging process, management of obesity, body-building or enhancement of athletic performance. It is hard to know how much of the multi-billion dollar global market for rhGH is directed to unapproved and illegal use. It is also unclear how much is authentic hormone. A study done in 2014 provided some insights into the online distribution of rhGH (73). The authors chose 17 internet sites advertising human growth hormone, ordered from 3 of these sites, described what they received and analyzed the products by capillary zone electrophoresis and electrospray ionization-mass spectrometry. Most sites did not require a prescription, displayed no medical information and lacked any regulatory body logo, address or phone number. The brands that were advertised were the familiar European brands. Products from the 3 sites arrived unrefrigerated, lacked instructions and materials for reconstitution and contained 35% to 50% of the stipulated amount of hormone. In the past 4 years, several Chinese products, including Jintropin, have been featured on internet sites. Without enforcement of laws concerning rhGH, the underground market will continue to grow.

Disclosure

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Standardization of Growth Hormone and Insulin-like Growth Factor-I Measurement

Noriyuki Katsumata, MD, PhD

Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan

Corresponding author: Noriyuki Katsumata, MD, PhD, Department of Molecular Endocrinology, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan, Tel: +81-3-3416-0181, Fax: +81-3-3416-2222, E-mail: katsumata-n@ncchd.go.jp

Abstract

Determination of serum levels of GH and IGF-I is crucial for the diagnosis and treatment of GH deficiency and disorders related to GH excess such as acromegaly and pituitary gigantism. However, significant discrepancies in measured GH values among the methods were observed around the world. In Japan, the Study Committee for GH and Its Related Factors of The Foundation for Growth Science standardized GH values measured with various commercially available GH assay kits by creating formulas to adjust them to their averages. The committee also established reference values for IGF-I in Japanese subjects at all ages from childhood to adulthood. Internationally, collaborators have been working on the harmonization of GH measurements.

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Key words: *GH, IGF-I, Radioimmunoassay, Enzyme immunoassay, Standardization*

Introduction

Proper determination of serum levels of GH and IGF-I is essential for the diagnosis and treatment of GH deficiency (GHD) and disorders related to GH excess such as acromegaly and pituitary gigantism (1,2). Determination of GH levels is also useful as an indicator of hypothalamic-pituitary function. However, considerable discrepancies in measured GH values among the methods were noticed worldwide (3). In Japan, the Study Committee for GH and Its Related Factors of The Foundation for Growth Science standardized GH values measured with various commercially available GH assay kits to address this problem. The committee also established reference values for IGF-I in Japanese subjects at all ages from childhood to adulthood. This review mainly summaries the efforts to standardize GH and IGF-I values by the committee in Japan.

Standardizing GH Measurements

Methods for Serum GH Determination

In clinical practice, determination of serum GH levels is necessary for the diagnosis and treatment of disorders related to GH deficiency or excess (1,2). GH levels are mainly measured in stimulation or inhibition tests for GH secretion, since they show marked diurnal variation. Immunological methods are usually employed to determine serum GH levels. Initially, serum GH levels were measured with a competitive radioimmunoassay (RIA) using polyclonal antibodies and radioisotopes. In recent years, sandwich assays using monoclonal antibodies as well as polyclonal antibodies and enzyme immunoassays utilizing enzymes instead of radioisotopes to label materials have been developed for serum GH determination (3).

Immunoassays for serum GH intrinsically encompass some variability in the determined values. The variability derives from several factors, including the diversity of circulating GH molecules such as 22K- and 20K-GHs; the presence of GH-binding protein (GHBP), the extracellular domain of GH receptor, which is proteolytically cleaved from the cell surface receptor and released into the blood stream; differences in the measurement principles such as competitive or sandwich-type; differences in the antibodies used such as polyclonal or monoclonal; differences in the epitope specificity or reactivity of these antibodies against diverse GH molecules; differences in conditions under which the antigen-antibody reaction occurs, such as an equilibrium or nonequilibrium state; effects of serum components other than GHBP; differences in the so-called matrix effect; and differences in the reference standards in measurements (3,4).

Variations in GH Measurements and Resultant Possible Problems

Competitive RIAs with polyclonal antibodies have been less associated with variations in GH measurements among GH assay kits. However, with the advent of sandwich assays using monoclonal antibodies, such variations have become serious problems (3,4). The measured GH levels in individual samples from the same patient could vary depending on the GH assay kits used (**figure 1**) (5,6), which might lead to discordant diagnoses and thus conflicting decisions in clinical practice. In other words, some patients who are diagnosed with GHD and indicated for GH therapy based on serum GH levels measured with one assay kit could be diagnosed as having normal GH secretion and might not be treated with GH when another assay kit is used to determine serum GH levels.

Standardization of GH Measurements in Japan

Since 1991, several types of GH assay kits have been marketed in Japan, including two types of RIA kits. The Study Committee for GH and Its Related Factors of The Foundation for Growth Science, Japan has measured serum GH levels in 60 samples using these commercially available kits every year. Historically, GHD had been diagnosed based on GH values measured with RIA. Thus, the committee used the averages of values obtained with the two RIA kits as the reference values, and developed correction formulae to adjust the GH values measured with individual assay kits to the reference values (5).

Since 1998, the committee has developed correction formulae with a Deming linear structural relationship model using the averages of the values obtained with all the commercially available GH assay kits as the dependent variables (***table 1***) (6,7).

Along with developing these correction formulae, the committee sought to identify the cause of variability in GH measurements among the assay kits (6). The committee prepared a reference standard by diluting 22K-rhGH, and measured GH content in the in-house reference standards supplied with the individual kits using the diluted 22K-rhGH as a unified standard. The obtained values were not consistent with the declared values in the kits, and differences in the potency of the reference standards were observed

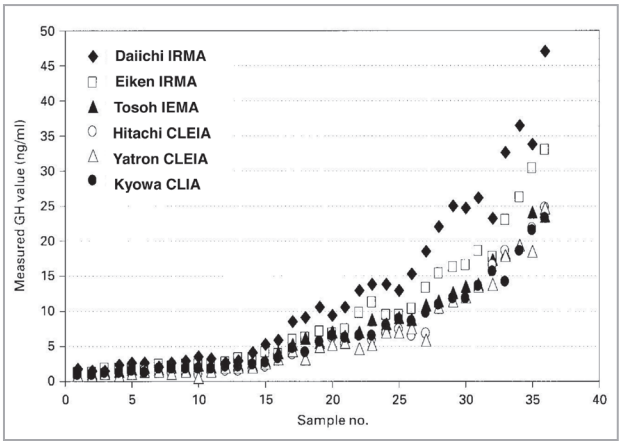

Figure 1. GH values measured according to kit standards
[adapted from Tanaka T, et al. Horm Res 2005;64(Suppl 2):6-11]
Note that measured GH values of individual samples vary widely depending on the kits. Daiichi IRMA, the immunoradiometric (IRMA) kit from Daiichi Radioisotope Labs Ltd, Tokyo, Japan; Eiken IRMA, the IRMA kit from Eiken Chemical Co. Ltd, Tokyo, Japan; Tosoh IEMA, the immunoenzymometric assay kit from Tosoh Co. Ltd, Tokyo, Japan; Hitachi CLEIA, the chemiluminescent enzyme immunoassay (CLEIA) kit from Hitachi Kasei Co. Ltd, Tokyo, Japan; Yatron CLEIA, the CLEIA kit from Yatron Co. Ltd, Tokyo, Japan; and Kyowa CLIA, the chemiluminescence immunoassay (CLIA) kit from Kyowa Medex Co. Ltd, Tokyo, Japan

Table 1. Correction formulae calculated using a linear structural relationship model to adjust the measured GH values (X) to their averages (Y) *[adapted from Tanaka T, et al. Horm Res 2005;64(Suppl 2):6-11]*

GH assay kit	Correction formula	Correlation coefficient
Daiichi IRMA	Y=0.64X+ 0.07	0.993
Eiken IRMA	Y=0.88X+0.02	0.997
Tosoh IEMA	Y=1.15X- 0.49	0.993
Hitachi CLEIA	Y=1.16X+1.05	0.983
Yatron CLEIA	Y=1.21X+0.47	0.993
Kyowa CLIA	Y=1.24X-0.30	0.994

Daiichi IRMA, the IRMA kit from Daiichi Radioisotope Labs Ltd, Tokyo, Japan; Eiken IRMA, the IRMA kit from Eiken Chemical Co. Ltd, Tokyo, Japan; Tosoh IEMA, the immunoenzymometric assay kit from Tosoh Co. Ltd, Tokyo, Japan; Hitachi CLEIA, the CLEIA kit from Hitachi Kasei Co. Ltd, Tokyo, Japan; Yatron CLEIA, the CLEIA kit from Yatron Co. Ltd, Tokyo, Japan; Kyowa CLIA, the chemiluminescence immunoassay kit from Kyowa Medex Co. Ltd, Tokyo, Japan.

among the kits (**figure 2**). Therefore, the discrepancies in GH measurements among the kits were considered to be at least in part due to differences in the potency of the supplied in-house reference standards (6). When GH levels in clinical samples were measured using the unified rhGH-based standard, the variations among the kits were reduced, and the obtained values decreased to 60% of the standardized values with the correction formulae (**figure 3**). Based on these observations, the committee requested kit suppliers to substitute the in-house GH standards with the reference standards based on 22K-rhGH.

Since 2005, the new reference standards based on 22K-rhGH have been included in all the GH assay kits available in Japan. As a result, the variations in GH measurements among the kits fell within the acceptable range, and therefore correction formulae were no longer needed. In addition, the cut-off value in the GH secretion stimulation test was set to 6 ng/mL rather than previous 10 ng/mL to diagnose GHD in children (6).

Recently, however, considerable variations in GH measurements among the GH assay kits were noticed again. Thus, in 2012, the committee reexamined the GH reference standards supplied with individual kits to address this problem (8). The committee prepared serial dilutions of the WHO International Standard GH (NIBSC Code 98/574) and quantified GH content in the diluted solutions with the GH assay kits. The observed values were almost equal to the theoretical values calculated from dilution factors in each kit, and did not differ among the kits (8). However, when GH levels were determined in serum samples, they differed significantly among the kits (**figure 4**). Therefore, the committee developed new correction formulae to avoid variability in measured values among the kits (8). In 2015, the committee confirmed the validity of the formulae developed in 2012 by measuring serum GH levels using the same assay kits (**figure 5**) (8).

International Approaches to Standardize GH Measurements

An international consensus statement on the standardization and evaluation of GH assays has been published (4). Collaborators have been working on the harmonization of GH measurements, which might include identification of a commutable matrix reference material and evaluation of liquid chromatography mass spectrometry as a reference measurement procedure (9). The importance of standardization, or at least harmonization, of GH assays was further endorsed strongly by the guidelines for GH treatment of the Pediatric Endocrine Society (10).

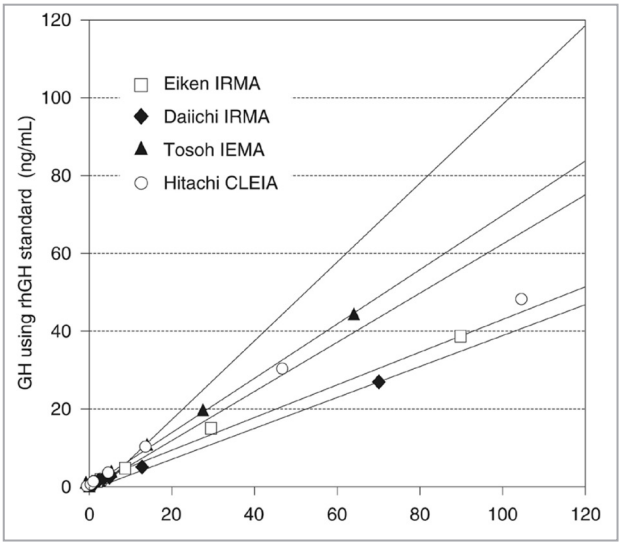

Figure 2. Relationship between declared kit standard values and their GH content measured according to rhGH standard
[adapted from Tanaka T, et al. Horm Res 2005;64(Suppl 2):6-11]
Daiichi IRMA, the IRMA kit from Daiichi Radioisotope Labs Ltd, Tokyo, Japan; Eiken IRMA, the IRMA kit from Eiken Chemical Co. Ltd, Tokyo, Japan; Tosoh IEMA, the immunoenzymometric assay kit from Tosoh Co. Ltd, Tokyo, Japan; Hitachi CLEIA, the CLEIA kit from Hitachi Kasei Co. Ltd, Tokyo, Japan

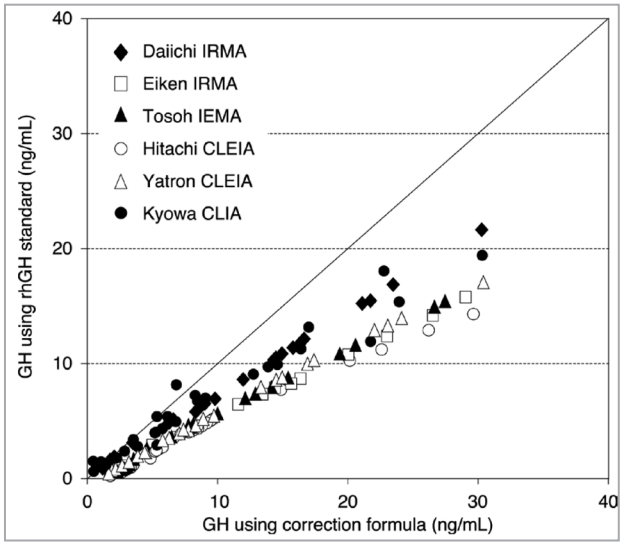

Figure 3. Relationship between GH values calculated with the correction formula and GH values measured with the rhGH standard
[adapted from Tanaka T, et al. Horm Res 2005;64(Suppl 2):6-11]
Daiichi IRMA, the IRMA kit from Daiichi Radioisotope Labs Ltd, Tokyo, Japan; Eiken IRMA, the IRMA kit from Eiken Chemical Co. Ltd Tokyo, Japan; Tosoh IEMA, the immunoenzymometric assay kit from Tosoh Co. Ltd, Tokyo, Japan; Hitachi CLEIA, the CLEIA kit from Hitachi Kasei Co. Ltd, Tokyo, Japan; Yatron CLEIA, the CLEIA kit from Yatron Co. Ltd, Tokyo, Japan; Kyowa CLIA, the chemiluminescence immunoassay kit from Kyowa Medex Co. Ltd, Tokyo, Japan.

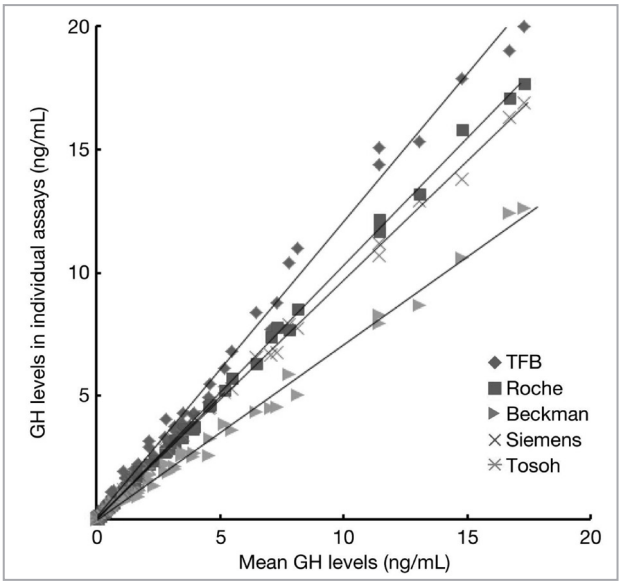

Figure 4. Relationship between mean and individual GH values measured by commercially available kits in the year 2012
(adapted from Katsumata N, et al. Endocr J 2016;63:933-936)
TFB, the IRMA kit from TFB, Inc, Tokyo, Japan; Roche, the ECLIA kit from Roche Diagnostics, Inc, Tokyo, Japan; Beckman, the CLEIA kit from Beckman Coulter, Inc, Tokyo, Japan; Siemense, the CLEIA kit from Siemens HCD, Inc, Tokyo, Japan; Tosoh, the IEMA kit from Tosoh Co., Ltd, Tokyo, Japan

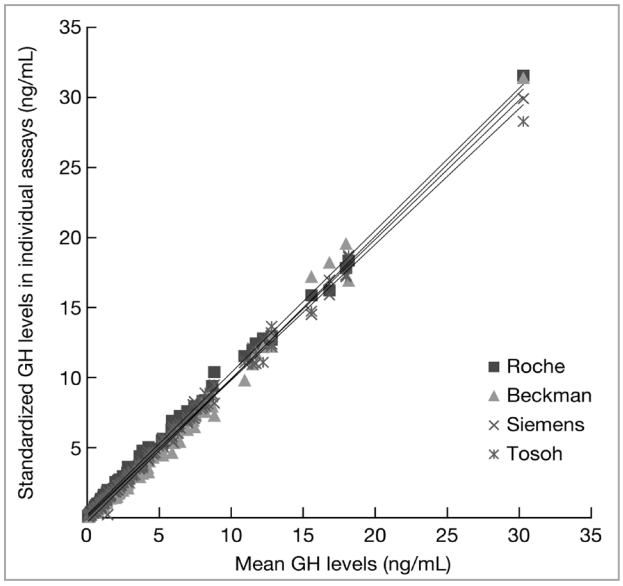

Figure 5. Relationship between mean and standardized GH values measured in the year 2015
(adapted from Katsumata N, et al. Endocr J 2016;63:933-936)
Roche, the ECLIA kit from Roche Diagnostics, Inc, Tokyo, Japan; Beckman, the CLEIA kit from Beckman Coulter, Inc, Tokyo, Japan; Siemense, the CLEIA kit from Siemens HCD, Inc, Tokyo, Japan; Tosoh, the IEMA kit from Tosoh Co., Ltd, Tokyo, Japan

Establishing IGF-I Reference Ranges

IGF-I Measurement

IGF-I is synthesized and secreted by tissues throughout the body such as the liver and bones in a GH-dependent manner. The majority of serum IGF-I is bound to IGF-binding proteins (IGFBPs). The half-life of circulating bound IGF-I is long, and serum IGF-I levels are less likely than GH to show diurnal variation. Therefore, serum IGF-I levels are used as an indicator of the kinetics of GH secretion. Serum IGF-I levels are usually measured by immunoradiometric assays without extraction after treatment with a diluent containing reagents that dissociate the binding of IGF-I to IGFBPs and inhibit its rebinding to IGFBPs (4).

Establishing IGF-I Reference Ranges for the Japanese Population

Serum IGF-I levels in healthy individuals vary with age, gender, pubertal stage, physiological condition, and ethnicity. Therefore, age- and gender-specific normative data of serum IGF-I levels for the Japanese population have to be established for their accurate evaluation (4). In 1996, Fujieda et al (11) and Shimatsu et al (12) measured serum IGF-I levels in Japanese children and adults, and proposed reference values for Japanese subjects. However, the reference values were categorized by age ranges, and thus occasionally resulted in problematic alterations in the standard deviation (SD) scores across the age ranges. In 2007, The Study Committee for GH and Its Related Factors of The Foundation for Growth Science investigated serum IGF-I levels in another Japanese cohort and reported IGF-I reference values for adults (13). In this study, the reference ranges were established using the LMS method and drawing smoothing curves for healthy adults 18 years of age or older (13). However, this report failed to provide proper IGF-I reference values in the transition period from puberty onward, during which IGF-I levels decline dramatically. To address this issue, the committee re-analyzed the previous data from children to adults with the LMS method, and generated smoothing curves for all ages by combining the IGF-I curves from 0 (neonate) to 25 years of age with the already established IGF-I curves for the older age groups (14). Evaluation of pretreatment IGF-I levels in patients with severe adult GHD of childhood onset using the newly generated IGF-I smoothing curves revealed a mean SD score of −4.9 ± 2.5. These results indicate that the newly established references are useful for screening severe adult GHD patients including those in the transition period (14).

Conclusion

Ideally, reference and standard values should be established independently for each GH assay kit. Practically, a lot of efforts have been made to standardize GH measurements internationally as well as in Japan. They include efforts to address enhancing the compatibility of GH measurements among kits, establishing correction formulae among kits, and developing so-called harmonization samples (4,9). Hopefully, international harmonization of GH measurements will be achieved in the near future, as discrepancies among kits confound both clinical practice and comparison of results across various research studies.

Disclosure

There are no competing interests to declare regarding this manuscript.

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Regulatory Role for Growth Hormone in Statural Growth: IGF-Dependent and IGF-Independent Effects on Growth Plate Chondrogenesis and Longitudinal Bone Growth

Francesco De Luca, MD

Division of Endocrinology and Diabetes, Children's Mercy Kansas City, Kansas City MO, University of Missouri School of Medicine, Kansas City, MO, USA

Corresponding author: Francesco De Luca, MD, Children's Mercy Clinics at Broadway, 3101 Broadway Boulevard, Kansas City, MO 64111, USA, Tel: 816-960-8964, E-mail: fdeluca@cmh.edu

Abstract

It was initially thought that the growth-promoting effects of GH were exclusively mediated by liver-derived Insulin-like Growth Factor-I (IGF-I). Subsequent studies demonstrated that GH promotes IGF-I synthesis and activity in other organs and in the growth plate.

GH has also IGF-I-independent growth-promoting effects. In Igf1 null mice, high circulating GH levels may be responsible for normal chondrocyte proliferation. Furthermore, tibial growth is reduced more in Ghr null mice than in Igf1 null mice, while the body of mice lacking both Ghr and Igf1 is smaller than that of mice lacking Igf1 or Ghr. The increased IGF-II expression in the growth plate in Igf1 null mice suggests that the IGF-I-independent effects of GH may be mediated by IGF-II. The effects of Igf1 receptor (Igf1r) gene deletion in chondrocytes indicate that GH may promote growth directly at the growth plate even when the local effects of IGF-I and IGF-II are abrogated.

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***Key words:** Growth Hormone, Insulin-like Growth Factor-I, Insulin-like Growth factor-II, Type 1 IGF Receptor, Growth plate, Chondrocyte*

Introduction

It is widely accepted that Growth Hormone (GH) is a major determinant of statural growth in mammals. GH deficiency and GH resistance in children reduce statural growth velocity and cause short stature, while GH excess leads to accelerated statural growth and gigantism. In addition, the growth-promoting effects of GH administration, first in animals then in humans, have been known for almost a century.

In this paper, we will discuss the historical journey during which the mechanistic role for GH in modulating statural growth has been progressively elucidated.

Biology of Statural Growth

Statural growth in humans results primarily by longitudinal bone growth, which in turn takes place at the growth plate (GP) through a process called endochondral ossification (1). The GP is organized into three distinct zones: the resting, the proliferative, and the hypertrophic zones. In the resting zone (the GP zone closest to the epiphysis), chondrocytes are embedded in cartilage matrix and proliferate rarely.

Farther toward the metaphysis, in the proliferative zone, the chondrocytes divide frequently and are arranged in columns. After having divided for a finite number of cell cycles, the proliferative chondrocytes at the bottom of each column stop replicating and enlarge to become hypertrophic chondrocytes. Hypertrophic chondrocytes differentiate and express alkaline phosphatase and type X collagen, and eventually undergo apoptosis. GP chondrocyte proliferation and hypertrophy, along with the extracellular matrix secreted by chondrocytes, lead to continued formation of new cartilage; this process is called chondrogenesis. While new cartilage is formed, the hypertrophic chondrocytes also release a variety of signaling molecules and enzymes. These signaling molecules promote vascular invasion from the metaphysis, which enables osteoclasts and osteoblast precursors to induce matrix degradation and remodeling of the hypertrophic zone into bone tissue. This process is called ossification.

The rates of GP chondrogenesis and longitudinal bone growth are regulated by multiple systemic (endocrine) factors, the most important of which are Growth Hormone, Insulin-like Growth Factors I and II (IGF-I and IGF-II), thyroid hormones, glucocorticoids, estrogens and androgens. On the other hand, the cellular processes responsible for chondrogenesis are also regulated by a network of local (paracrine) factors, expressed in the GP. These factors include several fibroblast growth factors (FGFs) and their receptors (especially FGFR3), bone morphogenetic proteins (BMPs), the parathyroid hormone-related protein (PTHrP) and its receptor (PTHR1), Indian hedgehog (IHH) pathway, and the C-type natriuretic peptide (CNP)-NPR2 pathway (1,2).

Growth Hormone

The GH molecule is a single-chain polypeptide consisting of 191 amino acids, and it is synthesized and secreted by somatrotroph cells in the pituitary gland. Although there are several circulating GH isoforms, the most abundant one is a 22-kDa monomer (3). GH synthesis and secretion are primarily regulated by two hypothalamic factors, GH releasing hormone (GHRH) and somatostatin. GH secretion is pulsatile and it is predominantly triggered by slow-wave sleep. Growth Hormone binding protein (GHBP) binds with high affinity to circulating GH and prolongs plasma GH half-life (4). The growth-promoting and metabolic functions of GH are mediated by the GH receptor (GHR), which belongs to the class I cytokine receptor superfamily. GHR is expressed abundantly in the liver, but it also is expressed in other tissues, including muscle, fat, and the growth plate (5,6). Once GHR is activated by GH, it initiates an intracellular signaling cascade, which includes the activation of a tyrosine kinase called JAK2 (7). JAK2 induces phosphorylation of transcription factors called STATs, which translocate to the nucleus and modulate the transcription of GH target genes. Abrogation of this signaling cascade is achieved by suppressor of cytokine signaling (SOCS) proteins, which induce proteosomal degradation of JAK2 (8).

IGF-I-Dependent Systemic Effects of GH

The first description of the systemic growth-promoting effects of GH dates back almost 100 years. In 1921, Evans and Long first demonstrated that bovine pituitary extracts injected in rats increased their body size (9). It was 37 years later that Raben described the growth-promoting effects of human pituitary GH extract in an adolescent boy, who experienced an accelerated statural growth rate after receiving multiple GH injections (10).

For many decades, it was thought that the growth-promoting effects of GH were exclusively indirect, i.e. only mediated by the liver-derived IGF-I. The concept of an exclusive indirect role for GH on growth regulation (i.e. fully mediated by liver-derived IGF-I) was the foundation of the original somatomedin hypothesis (11-13). However, subsequent studies have shown that the growth-promoting role of liver-derived IGF-I may actually be relatively marginal. LeRoith and colleagues created a mouse strain with liver-specific deletion of the Igf1 gene (LID mouse) (table) (14). The LID mice showed significantly reduced serum IGF-I levels (~25 % of the levels measured in wild-type [WT] mice). Yet, LID and WT mice did not differ in terms of femoral length. Of note, the LID mice exhibited a several-fold increase in circulating GH levels, which could be responsible for their preserved longitudinal bone growth. The same group subsequently developed a double mutant mouse strain by crossing mice lacking the GHR with Igf1 null mice carrying a hepatic Igf1 transgene (**table**) (15). Despite their normal serum IGF-I levels, the body growth of these double mutant mice was significantly retarded, suggesting that an IGF-I independent GH signal is essential for normal growth.

Local Effects of GH on Growth Plate Chondrogenesis and Longitudinal Bone Growth: IGF-I-Dependent and IGF-I-Independent Effects

In 1982 Isaksson *et al*. reported that GH injected into a rat tibial growth plate would promote longitudinal bone growth, and it would do so only in the injected bone (16). The direct effect of GH on bone is supported by additional evidence. IGF-I is expressed in the growth plate (17), and growth plate chondrocytes also express receptors for GH (18) and IGF-I (19). It has been demonstrated that systemic or local injection of GH stimulates the expression of IGF-I in the growth plate (20). This evidence has prompted a revised

Table. Mouse Models Exhibiting Deletion or Overexpression of the GH/IGF Signaling Pathway Genes

MOUSE MODEL	SERUM GH	SERUM IGF-I	BODY/BONE GROWTH	GROWTH PLATE	REFERENCE
LID liver-specific *Igf1* gene deletion	↑↑↑	↓↓	→	not described	14
GHRKO-HIT global *Ghr* gene deletion + hepatic *Igf1* transgene expression	not described	→	↓	not described	15
GHR -/- global Ghr gene deletion	not described	not described	↓↓	Narrowed. Reduced chondrocyte proliferation and hypertrophy	29
IGF-I -/- global Igf1 gene deletion	not described	not described	↓	Enlarged. Normal chondrocyte proliferation and reduced chondrocyte hypertrophy	29
***TamCart*Igf1r$^{-/-}$** Conditional cartilage-specific Igf1r gene deletion	→	→	↓ Body and bone growth were both increased by GH treatment	Narrowed. Reduced chondrocyte proliferation and hypertrophy. Growth plate height and chondrocyte proliferation and hypertrophy were all increased by GH treatment	49

↑, increased. →, unchanged. ↓, decreased. All comparisons are made with control mice.

somatomedin hypothesis, according to which GH can also modulate longitudinal bone growth via IGF-I expressed in extra-hepatic tissues, including the growth plate.

Experimental evidence has indicated that the effects of GH and IGF-I on bone growth are not completely overlapping. When they are injected together in hypophysectomized rats, they exert synergistic effects (21-23). In addition, GH administration to animals treated with maximal doses of IGF-I further enhances their growth (24).

Genetic manipulation in rodents has shed more light on IGF-I independent effects of GH on growth promotion. Transgenic mice overexpressing GH grow significantly more than their normal littermates (25,26), while mice overexpressing IGF-I do not (27,28).

Longitudinal bone growth is significantly more retarded by Ghr deletion than by Igf1 deletion (**table**) (29) and mice lacking both Ghr and Igf1 are smaller than mice lacking Igf1 or Ghr alone (30). In conclusion, several independent studies describing the effects of naturally occurring or experimentally obtained gene deletions in mice have demonstrated a more marked reduction of longitudinal bone growth in mice lacking GH activity than in mice with IGF1 gene deletion.

By studying the effects of GH and IGF-I in preadipocytes (31,32), and subsequent studies in cultured chondrocytes (33-37), it was postulated that GH and IGF-I act in the growth plate of the long bones on chondrocytes at different stages of maturation. According to the "dual effector hypothesis," GH would induce the generation of chondrocyte precursors in the resting zone, while IGF-I would stimulate further clonal expansion of chondrocytes at a later stage of development (proliferative chondrocytes) (31). However, this hypothesis has also been subsequently challenged. Wang *et al*. reported that the tibial growth plate resting zone is significantly enlarged in the Igf1 null mouse (29). Since GH levels are chronically elevated in these mice while IGF-I is completely absent from all tissues as well as from circulation, this observation provides indirect evidence for an IGF-I-independent role for GH in stimulating chondrocyte generation, in line with the dual effector theory. However, chondrocyte number and proliferation are normal in the Igf1 null mice growth plate, which does not completely fit with this theory. Ghr null mice demonstrate attenuation of both chondrocyte proliferation and hypertrophy, while Igf1 null mice exhibit only reduced hypertrophic chondrocyte size. Thus, these findings suggest that GH may have direct effects upon both chondrocyte generation and proliferation independent of IGF-I, while IGF-1 may affect only, or primarily, chondrocyte hypertrophy. The dual reduction in chondrocyte proliferation and hypertrophy can explain the more profound growth deficit in the Ghr null mouse than in the Igf1 null mouse.

Interestingly, IGF-II mRNA is increased in Igf1 null mice growth plates and reduced in Ghr null mice growth plates. It is therefore possible that GH may, in the absence of IGF-I, promote chondrocyte proliferation by stimulating Igf2 gene expression.

IGF-II has growth-promoting properties which are predominantly expressed prenatally. Mice with deletion of Igf2 exhibit growth retardation at birth (their birth size is

similar to that of Igf1 null mice) (38,39). When both Igf1 and Igf2 genes are deleted in mice, their birth size is more significantly reduced compared to that of mice with either Igf1 or Igf2 gene deletion (40), thus supporting a distinctive, rather than redundant, role for each of these two growth factors on fetal growth. Unlike Igf1 null mice, Igf2 null mice do not experience postnatal growth retardation. On the other hand, the expression of Igf2 is still abundant in the rodent growth plate in the first few weeks of postnatal life, to progressively diminish afterwards (6).

The type 1 IGF receptor, more commonly known as IGF1 receptor (IGF1R) binds both IGF-I and IGF-II with high affinity (41) and mediates their mitogenic and metabolic actions; in fact, monoclonal antibodies blocking the binding of IGF-I or IGF-II to IGF1R prevent the ability of both IGFs to stimulate cell replication (42,43). Deletion of Igf1r in mice leads to a 45% reduction of birth size, with 100% neonatal lethality (44). Concurrent deletion of Igf1 and Igf1r results in no further reduction of birth size compared to the deletion of Igf1r alone, thus indicating that virtually all the effects of IGF-I on body growth are mediated by the IGF1 receptor (45).

Expression of type 1 IGF receptor is abundant in all growth plate chondrocytes (6,46), and it is likely that both the effects of IGF-II on chondrocyte proliferation and of IGF-I on chondrocyte hypertrophy are mediated by this receptor.

Several patients with IGF1R gene mutations or deletions have been reported (47). Although the phenotype may vary, many of these patients exhibit severe intrauterine growth retardation and postnatal growth failure. In recent years, a number of children with IGF1R mutations have been treated with GH; in most of these children, GH induced a detectable, although modest, growth response (47).

Other Mediators of the direct GH Effect on Growth Plate Chondrogenesis and Longitudinal Bone Growth

To determine whether GH can affect growth plate chondrogenesis and longitudinal bone growth without the mediation of IGF-I or IGF-II, we used a transgenic mouse in which the Igf1r gene was conditionally ablated postnatally by Cre-lox recombination only in chondrocytes (48). Serum levels of GH and IGF-I were similar in chondrocyte-specific Igf1r null mice (KO) and control (C) mice. After 4 weeks, body and tibial growth of KO mice were significantly smaller than those of C mice. After 4 weeks of daily GH injections, body growth and tibial growth of GH-treated KO mice were significantly greater than those of untreated C or untreated KO mice. In the same study, untreated KO mice exhibited a reduced tibial growth plate height, which was due to reduced height of all three zones (epiphyseal, proliferative, and hypertrophic). GH treatment increased the height of all the zones in the growth plate.

GH was also able to stimulate both thymidine incorporation and collagen X mRNA expression in cultured chondrocytes transfected with IGF-1R siRNA. Thus, these findings suggest that GH has also IGF-independent effects on both chondrocyte proliferation and differentiation.

A possible mediator of this IGF-independent GH effect on growth plate chondrogenesis and bone growth, is NF-κB p65 (an intracellular transcription factor). GH activates NF-κB p65 in cultured chondrocytes, and the promoting effects of GH on chondrocyte proliferation and differentiation are diminished after silencing NF-κB p65 expression (49). GH also increased phosphorylation (i.e. activation) of NF-κB p65 in the growth plate of chondrocyte-specific Igf1r null mice (48). This proposed mechanism is supported in humans by the report of a child with GH insensitivity due to a mutation of the IκBκ gene (and secondary NF-κB p65 impaired action) (50).

BMP-2 is expressed in the growth plate and accelerates longitudinal bone growth by stimulating growth plate chondrocyte proliferation and chondrocyte hypertrophy (51). In our tamoxifen-inducible, cartilage-specific Igf1r knockout mice, systemic GH injections induced BMP-2 mRNA expression in the growth plate, suggesting that the GH-dependent increased expression of BMP-2 in the growth plate may be responsible, at least in part, for the IGF-independent, growth-promoting effects of GH.

Conclusions

A large body of evidence indicates that GH enhances statural growth postnatally through multiple mechanisms. It stimulates longitudinal bone growth indirectly by inducing the synthesis of IGF-I in the liver and other peripheral organs. As a result, once it is released, systemic IGF-I acts as an endocrine factor by reaching the growth plate of the long bones, where it stimulates growth plate chondrogenesis and longitudinal bone growth. On the other hand, GH induces IGF-I (and IGF-II) synthesis and activity at the growth plate, where IGFs function as paracrine growth factors. Furthermore, more recent evidence supports a direct (IGF-independent) regulatory role for GH in longitudinal bone growth. Indeed, in mice with a targeted deletion of the IGF1R in the growth plate (thus, with very marginal, if any, IGF-I and IGF-II activity) GH is still able to enhance growth plate chondrogenesis and longitudinal bone growth. Further studies are needed in order to better elucidate the molecular mechanisms through which GH can directly modulate statural growth.

Disclosure

The author has nothing to disclose.

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Genetic Mutations in the GH/IGF Axis

Sabina Domené, PhD, Horacio M. Domené, PhD

Centro de Investigaciones Endocrinológicas (CEDIE-CONICET), "Dr. César Bergadá", División de Endocrinología, Hospital de Niños R. Gutiérrez, Buenos Aires, Argentina

Corresponding author: Horacio M. Domené, PhD, Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE), CONICET – FEI – División de Endocrinología, Hospital de Niños "Ricardo Gutiérrez", Gallo 1330 – C1425EFD Buenos Aires, Tel: (+54-11) 4963-5931 int. 130, Fax: (+54-11) 4963-5930, E-mail: hdomene@cedie.org.ar, Web: www.cedie.org.ar

Abstract

The GH/IGF axis plays an important role in the control of pre and postnatal growth. At least 48 monogenic defects have been described affecting the production, secretion, and action of GH and IGFs.

*Molecular defects of the GH/IGF axis resulting in short stature were arbitrarily classified into 4 groups: **1**. Combined pituitary hormone deficiency (CPHD) (**a**. syndromic CPHD and **b**. non-syndromic CPHD), **2**. Isolated GH deficiency (IGHD), **3**. GH insensitivity, and **4**. IGF-I insensitivity.*

Genetic diagnosis is obtained in about 30-40% of children with growth retardation, severe IGHD, CPHD, apparent GH or IGF-I insensitivity, and small for gestational age. Increased accessibility to next generation sequencing (NGS) techniques resulted in a significant number of likely pathogenic variants in genes previously associated with short stature as well as in completely novel genes. Functional in vitro assays and in vivo animal models are required to determine the real contribution of these findings.

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***Key words**: Combined pituitary hormone deficiency, Isolated GH deficiency, GH insensitivity, IGF-I insensitivity, Genetic defects*

Introduction

The GH/IGF axis plays an important role in the control of pre and postnatal growth (1). While in the prenatal period, growth factors IGF-I and IGF-II are essential for longitudinal growth, mainly under the control of placental lactogen (PL) and nutritional resources (2), after birth pituitary GH becomes the predominant stimulator of IGF-I expression (3). From a historical point of view, in the 1920s and 1930s the growth promoting effect of pituitary extracts on rats (4) and the effect of the anterior pituitary on carbohydrate metabolism (5) were already recognized. However, much more research was still needed until human pituitary-extracted GH was available in 1958 to treat children diagnosed as GH deficient (GHD) based upon either clinical evaluation or bioassays measuring sulfation factor (6). It was not until the 1960s that a specific radioimmunoassay was available for the quantification of GH in serum samples (7) and the use of stimulation tests was a requirement to confirm the clinical diagnosis of GHD (8). The availability of recombinant human GH (rhGH) in 1985 opened up the possibility to potentially treat all patients diagnosed as GHD.

Although the pathogenic etiology of GHD is variable, and may result from different causes such as trauma, brain surgery, tumor, infection, radiation, and autoimmune diseases, it became clear that genetic defects in the GH/IGF axis also

could be involved in alterations in the production, secretion, and action of GH and IGFs. The first molecular diagnosis of complete GHD was reported in 1981 in three families with severe isolated GHD that developed anti GH-antibodies in high titer when treated with hGH (9). In this review, we describe the molecular defects of the GH/IGF axis resulting in short stature. We arbitrarily classified the genetic defects in the GH/IGF axis into 4 groups: **1.** CPHD (a. syndromic CPHD and b. non-syndromic CPHD), **2.** Isolated GH deficiency (IGHD), **3.** GH insensitivity, and **4.** IGF-I insensitivity (**figure 1**).

Because genetic causes of defects in the GH/IGF axis have been exhaustively reviewed elsewhere (10-15), we chose to focus mainly on the genetic aspects, such as mode of inheritance and type of mutations, as well as *in vivo* knockout models in both mice and zebrafish to compare the clinical human phenotype with other vertebrate models widely used to model human disease.

Generation of Animal Models for Endocrine Genetic Diseases using Gene-Targeting Techniques

Animal models of genetic diseases constitute an important tool to understand the function of individual genes, particularly when they are used to reproduce human inherited diseases (16). In the case of the GH-IGF axis, several mice strands presenting severe growth impairment were studied (17,18). Later, molecular studies demonstrated that they presented naturally occurring gene mutations in specific genes involved in the regulation of GH expression (19). The development of homologous recombination in embryonic stem cells (20,21) by using specific targets to disrupt gene sequences allowed the generation of null mutants, where a gene is disrupted by the introduction of a cassette carrying a positive selection marker, such as the neomycin resistance gene, under the control of a strong promoter (16,22). Soon it became clear that, even considering the anatomic and physiological differences between rodents and humans, single-gene-knockout (KO) mice may recapitulate some of the consequences of the lack of GH (GH-deficient mice) and the lack of GH action (GH-insensitivity mice) (23-26).

To further dissect the impact of the ablation of a gene in a specific tissue, Sauer and Henderson developed the Cre/loxP system (27), in which a targeted gene was flanked by two loxP sequences (a 34-base pair sequence) that are specifically recognized by Cre, a recombinase protein encoded by the coliphage P1. Recombination occurs specifically at the loxP sequences with loss of the sequence flanked by these two sites. While the gene of interest is flanked by loxP sites, a Cre protein is transfected by using a vector under the control of a tissue specific enhancer-promoter (for example, the albumin promoter to selectively disrupt gene expression in the liver). With this strategy, the expression of IGF-I was selectively disrupted in the liver by Cre-mediated site-specific recombination. This was a remarkable achievement that allowed the characterization of the impact of circulating IGF-I on postnatal growth (28).

More recently, a novel technique was developed that uses engineered nucleases such as clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein (cas)9 that generates a DNA double-strand break at the targeted genomic locus. In the absence of a template, it results in an insertion and/or deletion that disrupts the targeted locus. In the presence of a donor template, carrying the mutation of interest, the repair results in the inclusion of the designed mutation (29,30). This technique proved much more efficient than homologous recombination and has demonstrated some therapeutic applications (31).

The zebrafish (*Danio rerio*) is a vertebrate animal model which has many advantages compared to the mouse and is becoming an important model in translational research (32). Among the most important advantages are the transparency of the embryos (making it an ideal model for developmental biology), external fertilization, rapid development, and the large number of embryos obtained from each couple (ideal for high throughput analysis). Genetic manipulation is also relatively easy. Transient knockdown is achieved using morpholinos, which are synthetic oligonucleotides that either block translation by being complementary to the translational start site or block splicing by complentarity to the splicing sequence of the target mRNA (33). More recent knockout techniques, also applied in zebrafish, involve the use of CRISPR/Cas9, useful for obtaining stable transgenic lines with deletions or insertions (34).

Combined Pituitary Hormone Deficiency (CPHD)

Combined pituitary hormone deficiency (CPHD) or panhypopituitarism is characterized by the absence of GH and one or more other pituitary hormones (LH, FSH, PRL, TSH, and ACTH). Although as many as 30 genes have been found to be associated with CPHD, eight of them are the most frequently studied: *GLI2*, *HESX1*, *LHX3*, *LHX4*, *POU1F1*, *PROP1*, *OTX2*, and *SOX2* (12,13,35-37) (**table 1**).

Mutations in early transcription factors, such as *LHX3*, *LHX4*, *HESX1*, *GLI2*, *OTX2*, and *SOX2* that participate in pituitary ontogenesis, lead to syndromic CPHD where pituitary dysfunction is associated with craniofacial anomalies such as septo-optic dysplasia or holoprosencephaly (HPE). On the other hand, mutations in later-acting transcription factors involved in pituitary cell differentiation, such as POU1F1 and PROP1, lead to non-syndromic CPHD with a pituitary-specific phenotype and absence of craniofacial anomalies (13).

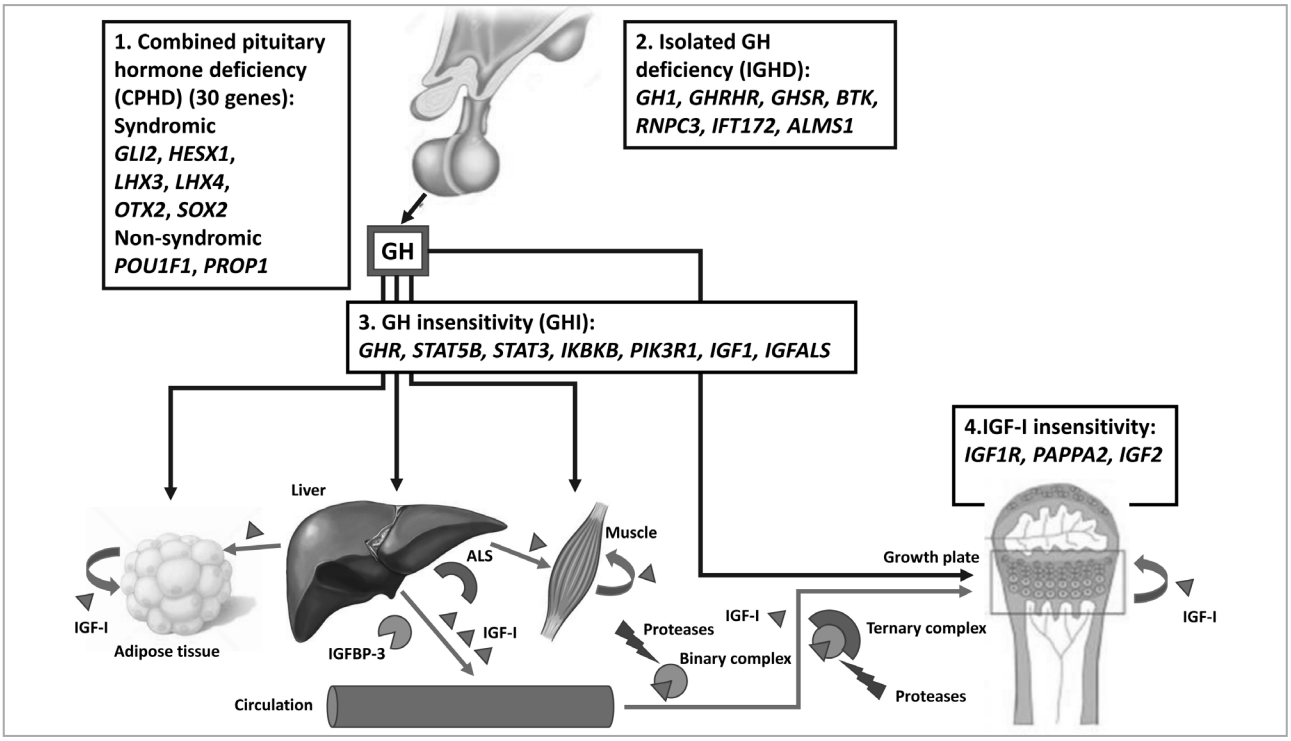

Figure 1. Monogenic defects in the GH/IGF axis

Genetic defects in early transcription factors that participate in the pituitary ontogenesis (LHX3, LHX4, HESX1, GLI2, OTX2, and SOX2, among others), result in syndromic combined pituitary hormone deficiency (CPHD), while gene mutations in those transcription factor that are expressed later during pituitary cell differentiation (POU1F1 and PROP1) result in non-syndromic CPHD.

Genetic causes of isolated GH deficiency (IGHD) include defects in the gene that encodes GH (GH1), and other genes that are involved in the synthesis and secretion of GH (GHRHR, GHSR, BTK, RNPC3, IFT172, ALSM1).

GH insensitivity can arise from molecular defects in the gene encoding the GH receptor (GHR) or in several other genes that participate in the signaling transmission of GH action (STAT5B, STAT3, IKBKB, IL2RG, PIK3R1), IGF-I synthesis (IGF1), or IGF-I transport (IGFALS).

Resistance to IGF-I occurs associated to gene defects in its own receptor (IGF1R), a specific protease (PAPPA2) or the gene encoding IGF-II (IGF2). Straight blue arrows indicate sites of GH action (liver, muscle, adipose tissue). Straight red arrows indicate the action of "endocrine IGF-I", mainly produced in the liver and circulating as free-IGF-I, forming binary complexes (associated to IGFBPs) or ternary complexes (associated to IGFBP-3, or -5 and ALS). Curved red arrows denote the action of "paracrine IGF-I" (acting near its site of production).

Appendix

Glossary of gene names

LHX3: Lim Homeobox Gene 3
LHX4: Lim Homeobox Gene 4
HESX1: Homeobox Gene Expressed in ES Cells
GLI2: Gli-Kruppel Family Member 2
OTX2: Orthodenticle, Drosophila, Homolog of, 2
SOX2: SRY-Box 2
POU1F1: Pou Domain, Class 1, Transcription Factor 1
PROP1: Paired-Like Homeobox 1
GH1: Growth Hormone 1
GHRHR: Growth Hormone-Releasing Hormone Receptor
GHSR: Growth Hormone Secretagogue Receptor
BTK: Bruton Agammaglobulinemia Tyrosine Kinase
IFT172: Intraflagellar Transport 172, Chlamydomonas Homolog of
ALMS1: Alms1 Gene
GHR: Growth Hormone Receptor
STAT5B: Signal Transducer and Activator of Transcription 5b
STAT3: Signal Transducer and Activator of Transcription 3
IKBKB: Inhibitor of Kappa Light Chain Gene Enhancer in B Cells, Kinase of, Beta
IL2RG: Interleukin 2 Receptor, Gamma
PIK3R1: Phosphatidylinositol 3-Kinase, Regulatory Subunit 1
IGF1: Insulin-Like Growth Factor I
IGFALS: Insulin-Like Growth Factor-Binding Protein, Acid-Labile Subunit
IGF1R: Insulin-Like Growth Factor I Receptor
IGF2: Insulin-Like Growth Factor II

Table 1. Combined pituitary hormone deficiency (CPHD)

Gene OMIM	Phenotype OMIM	Human phenotype	Mouse phenotype	Zebrafish phenotype
LHX3 600577	CPHD 3 221750	Absence of GH, PRL, TSH, LH, FSH, rigidity of the cervical spine	Die *in utero* or after 24 hours post birth, absence of GH, PRL, TSH, LH, FSH	Endocrine phenotype unknown
LHX4 602146	CPHD 4 262700	Variable GH, TSH, ACTH and LH, and FSH deficiencies, hypoplasia of the pituitary, poorly developed Sella-turcica	Absence of differentiation of the anterior pituitary cell lineages: GH, TSH, ACTH, LH, and FSH deficiencies	Endocrine phenotype unknown
HESX1 601802	GH deficiency with pituitary anomalies CPHD 5 Septo-optic dysplasia 182230	Optic nerve hypoplasia, pituitary hypoplasia, midline brain abnormalities	Anterior central nervous system defects and pituitary dysplasia	Wildtype
GLI2 165230	Culler-Jones syndrome 615849 Holoprosencephaly 9 610829	HPE, craniofacial abnormalities, hypopituitarism	Embryonically lethal	Reduced number of corticotrophs and increased number of lactotrophs
OTX2 600037	CPHD 6 613986	Anophthalmia or microphthalmia associated with pituitary hormone deficiency	Homozyogous knockout mice die during midgestation. Heterozygous mutant mice present eye, pituitary and craniofacial defects	Mild microphthalmia and shortening of the pharyngeal skeleton
SOX2 184429	Optic nerve hypoplasia and abnormalities of the central nervous system 206900	Anophthalmia or microphthalmia and hypoplastic anterior pituitary	Heterozygous mutants present reduced somatotroph number, GH content and reduction of pituitary size. Adult mouse presents GH, PRL, and TSH deficiencies	Shorter anteroposterior axis, smaller eyes and early lethality
POU1F1 173110	Pituitary hormone deficiency, combined, 613038	Pituitary hypoplasia and absence of GH, PRL and TSH	Snell mouse: growth insufficiency, infertility, hypothyroidism and deafness due to absence of GH, PRL, TSH, and gonadotropins	Severe dwarfism with absence of lactotrophs, somatotrophs, and thyrotrophs
PROP1 601538	Combined pituitary hormone deficiency 2 262600	Absence of GH, PRL, TSH, LH and FSH and pituitary hypoplasia	Ames mouse: absence of GH, PRL, TSH, LH and FSH and pituitary hypoplasia	Abnormal adenohypophysis and reduced expression of pou1f1, prl and gh

Syndromic CPHD

LHX3 gene (OMIM 600577), CPHD3 (OMIM 221750)

LHX3 is a member of the LIM homeodomain family of transcription factors which has a role in pituitary development and the organization of spinal cord neurons. Mutations in *LHX3* cause less than 1% of cases of CPHD and are characterized by absence of all anterior pituitary hormones except for ACTH and rigidity of the cervical spine (13). Some patients have sensorineural hearing loss. It has an autosomal recessive mode of inheritance and although there are reports of complete deletions of the gene, most mutations are missense or nonsense. *LHX3* gene mutations were first reported by Netchine *et al*. in two unrelated families where affected subjects presented severe growth retardation and GH, TSH, PRL, LH, and FSH deficiencies (38). Affected subjects also presented a rigid cervical spine leading to limited head rotation. These patients were homozygous for a missense mutation (p.Tyr116Cys). Homozygous mutant mice for *Lhx3* die *in utero* or within 24 hours post birth. Although Rathke's pouch forms, it fails to grow and differentiate, resulting in the absence of the anterior and intermediate lobes of the pituitary and affecting the determination of all pituitary cell linages except the corticotrophs, similar to the human clinical phenotype (39-41).

Endocrine defects in zebrafish have not yet been characterized in lhx3 morphants. Nevertheless, lhx3 morphants zebrafish embryos show motoneuron alteration (42).

LHX4 gene (OMIM 602146), CPHD4 (OMIM 262700)

LHX4 also encodes a LIM homeodomain transcription factor implicated in pituitary development and the organization of spinal cord neurons. Mutations in *LHX4* are responsible for less than 1% of CPHD cases which manifest with variable GH, TSH, ACTH and gonadotrophin deficiencies, hypoplasia of the pituitary and a poorly developed sella turcica (13). Mutations are mostly missense and deletions which are inherited in an autosomal dominant fashion with incomplete penetrance. One homozygous missense variant (p.Trh126Met) is associated with a lethal phenotype (43). A germline splice-site mutation in the *LHX4* gene was first reported by Machinis *et al* in 2001 in a family where affected members presented short stature, pituitary and hindbrain defects, and abnormalities of the sella turcica. The intronic mutation has a dominant pattern of inheritance (44). Clinical characteristics of patients with LHX4 mutations have been reviewed recently (45). Mice deficient of *Lhx4* do not undergo differentiation of the anterior pituitary cell lineages (40). As in *lhx3*, endocrine consequences of the absence of *lhx4* have not been studied in zebrafish yet.

HESX1 gene (OMIM 601802), GHD with pituitary anomalies, CPHD5, and septo-optic dysplasia (OMIM 182230)

HESX1 is a member of the paired-like class of homeobox transcription factors with a crucial role in the formation of the pituitary and forebrain. *HESX1* mutations are responsible for less than 1% of cases in which CPHD is associated with optic nerve hypoplasia, pituitary hypoplasia, and midline brain abnormalities (13). Dattani *et al*. reported two siblings with septo-optic dysplasia (SOD) homozygous for a p.Arg53Cys missense mutation within the HESX1 homeodomain which destroyed its ability to bind target DNA (46). However, *HESX1* mutations are not a common finding in patients with SOD since sequencing of this gene in 228 patients presenting congenital pituitary defects (IGHD or SOD with panhypopituitarism) identified only three different heterozygous missense mutations in three patients with mild pituitary hypoplasia or SOD (47). Autosomal dominant with incomplete penetrance as well as autosomal recessive patterns of inheritance have been reported (48). Hesx1 null mice exhibited anterior central nervous system defects and pituitary dysplasia similar to the phenotype observed in humans (46). Although hesx1 knockdown had no effect on zebrafish, Andoniadou *et al*. showed that injecting a hesx1 morpholino into a 'sensitized' headless (*tcf3*) zebrafish mutant leads to severe forebrain and eye defects, suggesting an interaction between hesx1 and the wnt pathway in zebrafish (49).

GLI2 gene (OMIM 165230), Culler-Jones syndrome, (OMIM 615849) holoprosencephaly 9 (OMIM 610829)

The *GLI2* gene is a transcription factor involved in the Sonic Hedgehog (SHH) pathway. GLI2 mutations were first identified in patients with holoprosencephaly (HPE) (50). HPE is characterized by defects in forebrain cleavage which include defective anterior pituitary formation and panhypopituitarism. HPE can also be caused by mutations in *SHH, PTCH1, TGIF, SIX3, ZIC2, NODAL, FOXH1, CDON, FGF8*, and *DISP1* (51-54). GLI2 molecular defects are inherited in an autosomal dominant pattern with incomplete penetrance and variable phenotype, and the most common mutations are frameshift, nonsense and missense heterozygous mutations. By screening 390 unrelated patients, Roessler *et al* identified heterozygous truncating mutations in the *GLI2* gene caused by non-sense (p.Trp113*; p.Arg168*), frameshift (c.2274del1), and splice-site (IVS5 + 1G>A) mutations in four families. Clinical features included defective anterior pituitary formation and panhypopituitarism, with or without overt forebrain cleavage abnormalities, and HPE-like midfacial hypoplasia (50). Mutations in GLI2 occur in 1.5 % of CPHD cases (13). Gli2 knockout mice die embryonically, and conditional knockout in Rathke's pouch showed that it is necessary for pituitary progenitor specification, proliferation, and differentiation (55). Zebrafish knockdown studies show important differences with mice and humans. While in all other vertebrates, *Gli2* is the main activator of Shh signalling and *Gli1* is a minor one, in zebrafish it is the opposite; *Gli1* rather than *Gli2* is the main activator of Shh signalling (56-58). Zebrafish have two *gli2* genes (*gli2a* and *gli2b*) and knockdown of both using morpholinos affected the endocrine cell position in the pituitary, reduced the number of corticotrophs and increased the number of lactotrophs (59).

OTX2 gene (OMIM 600037), CPHD 6 (OMIM 613986)

OTX2 is a gene encoding a member of the homeobox transcription factor that is involved in the development of the brain and head structures (11). Heterozygous missense gene mutations were reported in 2 patients presenting GH, TSH, LH, FSH, and ACTH deficiencies. Magnetic resonance imaging revealed anterior pituitary hypoplasia with an ectopic posterior pituitary (60). Both patients presented the same missense mutation in the *OTX2* gene (p.Asn233Ser). *In vitro* studies showed that while wildtype and mutant OTX2 protein bound equally well to two specific sites in the 5-prime flanking region of the *HESX1* gene, mutant *OTX2* revealed decreased transactivation, resulting in a dominant negative inhibitor of *HESX1* gene expression. While homozyogous *Otx*-knockout mice die during midgestation, Otx heterozygous mutant mice present eye, pituitary and craniofacial defects (61,62). In zebrafish, morpholinos targeting otx2 result in mild microphthalmia and shortening of the pharyngeal skeleton at 5 days post fertilization (dpf) (63).

SOX2 gene (OMIM 184429), Optic nerve hypoplasia and abnormalities of the central nervous system (OMIM 206900)

SOX2 (together with SOX1 and SOX3) is a member of transcription factor related to SRY family that is expressed in many different embryonic tissues involved in the development of brain, pituitary, and otic and nasal placodes (64). Submicroscopic deletions and truncating mutations in the *SOX2* gene were identified in a small number of individuals with anophthalmia (65). In humans, the clinical presentation of patients with *SOX2* mutations includes hypogonadotrophic hypogonadism and CPHD. Most of the affected cases presented anophthalmia/microphthalmia, developmental delay, short stature, and male genital tract abnormalities. Patients presented heterozygous de novo or inherited *SOX2* mutations. They also may present other anomalies like anterior pituitary hypoplasia, defects of the corpus callosum, learning difficulties, sensorineural hearing loss, and esophageal atresia. Some of the mutations result in truncated protein products, exhibiting partial or complete loss of function (DNA binding, nuclear translocation or transactivation). Heterozygous loss of function of *Sox2* in the mouse is associated with a reduction in somatotroph number and GH content, as part of a general reduction in pituitary size. It was also shown that the adult mouse presents low circulating levels of GH, PRL, and TSH (66). Zebrafish sox2 morphants have a shorter anteroposterior axis and smaller eyes in addition to early lethality (5 dpf) compared to wildtype controls (67).

Non-Syndromic CPHD

POU1F1 gene (OMIM 173110), CPHD1 (OMIM 613038)

The transcription factor POU1F1 (previously known as Pit1) belongs to the POU family of transcription factors and is essential for the differentiation of somatotrophs, lactotrophs, and thyrotrophs. *POU1F1* gene is specifically expressed in the developing pituitary before the differentiation of somatotrophs, lactotrophs, and thyrotrophs and plays an important role in the differentiation of these cell linages as well as in the transcriptionally regulated expression of GH, PRL, and TSH. *POU1F1* mutations account for about 2.8 % of cases of CPHD with pituitary hypoplasia and absence of GH, PRL and TSH (13). In 1992, four independent groups (68-71) identified homozygous or heterozygous gene mutations in the POU1F1 gene (p.Arg172*, p.Arg271Trp, Ala158Pro) in patients with CPHD. In homozygous patients, POU1F1 mutations were loss-of function, while in patients presenting heterozygous mutations, the mutant protein retained the ability to bind DNA but lost its transcriptional activity, presenting a dominant-negative effect. Its inheritance is usually autosomal recessive, but it can be autosomal dominant for dominant negative mutations such as p.Arg271Trp. Missense, nonsense, splicing, and frameshift mutations have been reported. Interestingly, the Snell mouse has a mutation in the *Pou1f1* gene (*Pou1f1*$^{dw/dw}$, p.Trp251Cys) and it exhibits growth insufficiency, infertility, hypothyroidism and deafness, with absence of GH, PRL, TSH, and gonadotropins (72-74). Zebrafish knockdown of *pou1f1* shows severe dwarfism with absence of lactotrophs, somatotrophs and thyrotrophs (75).

PROP1 gene (OMIM 601538), CPHD2 (OMIM 262600)

Prop1 is a paired-like homeodomain transcription factor involved in the development of somatotrophs, lactotrophs, thyrotrophs, and gonadotrophs. Wu *et al* (76) identified homozygous or compound heterozygous mutations in the *PROP1* gene in four CPHD families, in which affected patients presented GH, PRL, TSH, LH, and FSH deficiencies. Mutations in *PROP1* are the most common cause of CPHD (representing up to 15 % of cases) and cause CPHD with pituitary hypoplasia and absence of GH, PRL, TSH, LH, and FSH (13). Its inheritance is autosomal recessive, and the most recurrent mutations are 301-302delAG and 150delA which have been found to be founder variants (77). The Ames dwarf mutant mouse is *Prop1* deficient and shows the same phenotype as patients with *PROP1* mutations (78,79). Knockdown of *prop1* in zebrafish embryos using morpholinos showed abnormal adenohypophysis morphology with reduced expression of *pou1f1*, PRL and GH (80). Interestingly, expression of lhx3 was also diminished.

Many other genes have been found to cause CPHD, but they are well beyond the scope of this review (for a more detailed description see reference 13).

Isolated GH Deficiency (IGHD)

Growth hormone deficiency (GHD) is a relatively common disorder, occurring in 1 out of 4,000 to 10,000 live births (81). Most frequently, it occurs as a sporadic condition of unknown etiology (82) but severe forms of isolated GHD (IGHD) may have a genetic basis (83). The diagnosis of GHD in childhood is based on auxological assessment, radiological evaluation, and biochemical tests. The diagnosis of isolated GHD requires the characterization of normal function of other pituitary hormones including TSH, ACTH, PRL, LH, FSH, and ADH. However, since a progressive compromise of pituitary hormones has been reported in children previously diagnosed as GHD, this diagnosis is often a provisional one (84,85) and systematic follow-up of these patients is mandatory to identify those subjects that develop additional pituitary hormone deficiencies. Familial IGHD has been associated with four Mendelian disorders (86-88), including two autosomal recessive (Type IA and IB), one autosomal dominant (Type II), and one X-linked (Type III) form (**table 2**).

Table 2. Isolated GH deficiency (IGHD)

Gene OMIM	Phenotype OMIM	Inher.	Human phenotype	Mice phenotype	Zebrafish phenotype
GH1 139250	Type IA complete GHD 262400	AR	Ab formation on GH treatment	Dwarf phenotype	vizzini mutant: severe growth retardation, small body size and increased accumulation of adipose tissue
GH1 139250	Type IB GHD 612781	AR	Immune tolerance to GH treatment. Low but detectable GH	Idem	Idem
GH1 139250	Type II GHD 173100	AD	Variable severity of GHD, potentially evolves to MPHD	idem	Idem
GH1 139250	Kowarski syndrome 262650	AR	Bioinactive GH	Idem	Idem
GHRHR 139191	Type IB GHD 612781	AR	Immune tolerance to GH treatment. Low but detectable GH	Little mouse: reduced GH secretion and a dwarf phenotype	Unknown
GHSR 601898	Partial GHD 615925	AR AD	Partial GHD	Serum IGF-I levels and body weight are modestly reduced, less appetite and adiposity compared to wildtype.	Unknown
BTK 300300	Agammaglobulinemia and GHD 307200	XLR	Type III, hypogammaglobulinemia	Xid mouse: impairment of peripheral B cell maturation	Severe anterior truncation of embryos (dorsalization)
RNPC3 -	-	AR	Severe GHD, pituitary hypoplasia	Unknown	caliban mutant: arrested development of digestive organs (intestine, liver and pancreas)
IFT172 607386	Short-rib thoracic dysplasia 10 with or without polydactyly 615630	AR	Growth retardation, pituitary hypoplasia, and ectopic posterior pituitary	Wimple mouse: altered left-right patterning	Ventral body-axis curvature, formation of renal cysts and cartilage defects
ALMS1 606844	Alstrom syndrome 203800	AR	Reduced GH reserve	Obesity, hypogonadism, hyperinsulinemia, retinal dysfunction, and late-onset hearing loss	Reduced beta-cell production

***GH1* gene (OMIM 139250), Type IA GHD (OMIM 262400)**

Type IA IGHD was first described by Illig *et al*. (89) in 1970 in three Swiss siblings with severe short stature, early growth retardation, extreme dwarfism in adulthood, and a characteristic phenotype. These patients developed high titers of anti-GH antibodies, which arrested their growth response to pituitary-extracted GH treatment. However, it was not until 1981 that the etiology of this condition was resolved (9). Genomic DNA samples were enzymatically digested using endonucleases and DNA fragments characterized by Southern blot using ^{32}P-labeled hGH cDNA sequences as probes. A homozygous deletion of about 7.5 kb, including the *GH1* gene, was found in four affected subjects from three different families. This work could be considered the first genetic characterization of a molecular defect in the GH-IGF axis. Although most of the patients presenting relatively large deletions (including the *GH1* gene) develop anti-GH antibodies preventing a growth response when treated with hGH, preservation of a growth response has been reported in some patients despite their high titer of anti-GH antibodies (90). In addition, some patients harboring *GH1* gene deletions do not develop anti-GH antibodies (91).

To date, deletions of different sizes (6.7, 7.0, 7.6, 45 kb, double deletions) within the GH-gene cluster have been characterized as molecular defects in IGHD (92-95), with the 6.7 Kb deletion being the most frequent (70-80%). Although most of the patients are homozygous for a specific deletion, and the parents heterozygous for the same genetic defect, some compound heterozygous cases with one deleted and one mutated allele or two different *GH1* gene deletions have been reported in non-consanguineous families (96-99). Small deletions and even a single amino acid substitution can be the cause of isolated Type 1 GH deficiency (100,101).

Mouse models of the disease include a dwarf phenotype observed when somatotrophs were genetically ablated (102). Finally, a zebrafish mutant with a stop codon mutation in the *gh1 gene*, called *vizzini*, was identified in 2013 which displayed severe growth retardation and small body size compared to wildtype fish (103). This mutant also had increased accumulation of adipose tissue which was expanded at maturity.

GH1 gene (OMIM 139250), GHRHR gene (OMIM 139191), Type IB IGHD (OMIM 612781)

Patients with Type 1B IGHD are characterized by low but detectable circulating GH levels and short stature. Because these patients do not develop neutralizing anti-GH antibodies, they retain the capability to display growth acceleration when treated with rhGH (104). They present an autosomal recessive pattern of inheritance and the clinical phenotype is more variable than that observed in Type 1A. Some patients resemble those with Type 1A, presenting early postnatal severe growth retardation, whereas in other cases growth failure is only evident later in childhood. All these observations suggest that more than a single gene could be responsible for this alteration. Indeed, defects in 2 genes have been reported to cause GHD Type 1B: *GH1* and *GHRHR*. While mutations in the *GH1* gene are usually splice site mutations (105), non-sense and missense mutations in the *GHRHR* gene also have been found in patients with Type IB IGHD (106). The little mouse, a spontaneous mutant mouse, presents severe growth retardation, an autosomal recessive pattern of inheritance, and diminished secretion of GH and IGF-I, which resembles the clinical phenotype of patients with Type IB IGHD. In 1993, analysis of the *GHRHR* gene in this mouse revealed a single substitution at codon 60 (p.Asp60Gly) that resulted in complete absence of binding of *GHRHR* for its ligand (107,108). Three years later, a non-sense mutation in the *GHRHR* gene was described in two cousins from a consanguineous Indian family (109). Two large kindreds presenting *GHRHR* gene defects have been described: **a.** eighteen IGHD subjects from the Pakistani province of Sindh all presented the same nonsense mutation (p.Glu72*) (110,111) and **b.** a cohort of 105 individuals from the rural county of Itabaianinha, in the northeastern Brazilian state of Sergipe, presented a transversion (c.57+1G>A) in the consensus GT of the 5´splice donor site of intron 1 of the *GHRHR* gene (112,113). This mutation leads to the retention of intron 1 and the appearance of a premature stop codon 213 bases downstream. These subjects exhibit reduced GH responsiveness to stimulatory tests, reduced levels of IGF-I, IGFBP-3 and ALS, and anterior pituitary hypoplasia (for a detailed review see reference 114). To date, no zebrafish knockdown studies have been performed in the *GHRHR* gene.

GH1 gene (OMIM 139250), Type II IGHD (OMIM 173100)

This autosomal dominant IGHD constitutes the more frequent genetic alteration in the GH1 gene (106). Most of the mutations affect the first six base-pairs of intervening sequence 3 (5´IVS-3) (106,115,116), resulting in misssplicing at the mRNA level and the subsequent loss of exon 3. This alteration results in the production of a 17.5 kb GH isoform (117). Mutations have also been reported in exon 3 splice enhancer 1 (ESE1) as well as ESE2 (118). These mutations also result in increased levels of exon 3 skipped transcripts (119,120). This GH isoform lacks amino acids 32-71 and exhibits a dominant-negative effect on the secretion of the 22-kDa isoforms. The 17.5 kDa GH isoform is initially retained in the endoplasmic reticulum, disrupting the Golgi apparatus, and thus impairing the normal trafficking of the 22-kDa GH (121). Transgenic mice overexpressing the 17.5 kDa isoform exhibit a defect in the maturation of the GH secretory vesicles and present anterior pituitary hypoplasia due to loss of the majority of somatotrophs (122,123). Patients affected with Type II IGHD present a variable degree of growth retardation, probably reflecting the variable degree of exon 3 skipping (82). *In vitro* experiments have demonstrated that in a pituitary cell line, the expression of the 17.5-kDa-mutant GH induced endoplasmic reticulum stress and apoptosis, contributing to the decrease in wildtype GH secretion (124). The relative amount of the 17.5 kDa to 22 kDa hGH isoform could determine the impact on pituitary size, the severity of GHD, and the appearance of other pituitary hormone deficiencies (125,126). For this reason, these patients should be carefully followed for the early detection and replacement of other hormonal deficiencies.

A recurrent missense mutation in the GH1 gene also results in IGHD Type II. The p.Arg183His (p.Arg209His according to the novel nomenclature) GH1 gene mutation, characterized in more than 50 subjects worldwide, results in large phenotypic variability, ranging from normal stature and GH secretion to severe GHD (127,128). Accordingly, in ten affected subjects from three unrelated families followed in our hospital, we have found a large variability in height SDS among untreated affected individuals, with adult heights ranging from -5.41 to -2.28 SDS (unpublished data). The biological mechanism

by which this heterozygous mutation results in a functional deficient state has not been completely elucidated. The mutant p.Arg209His-GH appears to fold properly and has full bioactivity, but after packaging into secretory granules it is poorly secreted, presenting a dominant-negative effect on the secretion of the WT-GH (127).

BTK gene (OMIM 300300), Type III IGHD (OMIM 307200)

Type III IGHD is an X-linked recessive condition in which affected patients present deficiency of both GH and immunoglobulin (129,130). Mutations and/or deletions in the long arm of chromosome X could be responsible for this alteration. In addition, an intronic point mutation (c.1882+5G>A), leading to exon-skipping and a premature stop codon in the BTK gene, has also been reported to be responsible for this disease (131). The xid or X-linked immunodeficiency mouse has a missense mutation in the Btk protein (132). Btk deficiency in the mouse is associated with an impairment of peripheral B cell maturation, without a major early B cell developmental block (133). In zebrafish, knockdown of btk gene using a splicing morpholino leads to severe anterior truncation of embryos (dorsalization) and this was shown to occur through an increase in wnt-beta-catenin signaling evidencing BTK as a negative regulator of this signaling pathway (134).

GH1 gene (OMIM 139250), Bioinactive GH (OMIM 262650)

Short stature associated to a bioinactive GH was first proposed by Kowarski *et al* (135) in two short boys presenting normal stimulated GH and low somatomedin/IGF-I levels. They responded normally to acute and chronic rhGH administration by increasing IGF-I levels and growth velocity. Several years later, patients with normal levels of GH and short stature were found to have heterozygous *GH1* gene mutations. Arg77Cys-GH not only failed to stimulate tyrosine phosphorylation in IM-9 cells but also inhibited the ability of wildtype GH to stimulate phosphorylation, thus having a dominant negative action (136). The *GH1* mutation p.Asp112Gly results in a protein that, when associated to GHBP, preferentially forms GH-GHBP complexes with a 1:1 ratio, instead of the normal 1:2 ratio produced by wild type GH. This mutant GH was less capable of phosphorylating tyrosine residues in GHR, JAK2, and STAT5 in IM-9 cells compared to wildtype GH (137). These two GH-mutant proteins with reduced or absent bioactivity, probably impaired the wildtype GH action and therefore could be responsible for the short stature observed in these patients.

GHSR gene (OMIM 601898), isolated partial GHD (OMIM 615925)

Isolated partial GHD (GHDP) can also be caused by heterozygous, compound heterozygous or homozygous mutations in the growth hormone secretagogue receptor gene (*GHSR*). In 2006, Pantel and colleagues (138) described homozygous and heterozygous p.Ala204Glu mutations in two probands from two unrelated Moroccan families. Short stature was present in some but not all heterozygous carriers' relatives, indicating incomplete penetrance and variable expressivity. Functional *in vitro* studies indicate that the mutant *GHSR* presented decreased cell surface expression and lacked constitutive activity of the receptor, while preserving its ability to respond to ghrelin, its natural ligand. Subjects carrying GHSR mutations present a clinical and biochemical phenotype of partial GHD or idiopathic short stature (139). Three different mouse models with *GHSR* deficiency have been reported in the literature. In two of them, *GHSR* gene has been removed by homologous recombination of mouse embryonic stem (ES) cells (140,141) while the other had the *GHSR* locus modified by the insertion of a loxP-flanked transcription blocking cassette (142). Although the Ghsr-null mice are not dwarf, serum IGF-I levels and body weight are modestly reduced compared to wildtype littermates (140,142). Despite this modest impact on postnatal growth, the major impact of GHSR deficiency seems to be related to some protection against diet-induced obesity (142). There is no known zebrafish knockdown of this gene yet.

RNPC3 gene

A novel monogenic defect resulting in severe IGHD has been reported (143) in three sisters born with normal length to normal statured and non-consanguineous parents. The patients showed severe postnatal growth retardation (height -5.0 to -6.6 SDS at diagnosis), typical physical features of GHD including delayed bone maturation, mild microcephaly and normal development. GH levels after standard stimuli and basal IGF-I and IGFBP-3 levels were almost undetectable. They presented a good response to therapeutic rhGH replacement. RT-PCR indicated normal amount and sequence of *GH1* gene transcripts. Whole exome sequencing (WES) analysis of one proband revealed a missense (c.1320C>A, p.Pro474Thr) and a nonsense (c.1504C>T, p.Arg502*) mutation in the *RNPC3* gene. Sanger sequencing validated that the three affected sisters are compound heterozygous for both mutations. This gene encodes a 65K protein that is a component of the U12-type spliceosome. Two types of spliceosomes catalyze splicing of pre-mRNAs. The major U2-type spliceosome is found in all eukaryotes and removes more than 99% of pre-mRNA introns. The minor U12-type spliceosome is found in some eukaryotes, is rare and has distinct splice consensus signals. The p.Pro747Thr mutation alters a highly conserved proline residue located in a turn position between β-3-strand and α-2-helix. Such turn positions are typically non-replaceable by other amino acids. In addition to mRNA instability due to non-sense mediated RNA decay (NMD), the p.Arg502* mutation deletes the last 15 C-terminal residues that are highly conserved.

No mouse model for Rnpc3 gene knockout has been reported yet. Zebrafish *rnpc3* mutant *caliban*, identified in an ethylnitrosourea (ENU) mutagenesis screen, shows arrested development of digestive organs, intestine, liver, and pancreas at 120 hours post fertilization (hpf) and these embryos die between 7- 10 dpf. These embryos also show delayed yolk resorption and smaller eyes (144).

IFT172 gene (OMIM 607386), short-rib thoracic dysplasia 10 with or without polydactyly (OMIM 615630)

A single case of functional GHD caused by compound heterozygous mutations in the *IFT172* gene (a missense mutation p.Cys1727Arg and a splice site mutation c.337-2A>C) have been reported in a boy with growth retardation, pituitary hypoplasia, and ectopic posterior pituitary (145). Although mutations in this gene, important for ciliary function, have been previously described in retinitis pigmentosa and short-rib thoracic dysplasia, the interaction between the protein coded by the *IFT172* gene with LHX3 and LHX4 could indicate a role for this gene in pituitary development (146). The mouse null mutant *wimple* has loss of motor neuron specification in the ventral neural tube and defects in left-right patterning. Both are due to a loss of hedgehog signaling (147). Zebrafish morphants have ciliopathy phenotypes, including ventral body-axis curvature, formation of renal cysts and cartilage defects which resemble the human phenotype (148). They also show hydrocephaly, an altered cranial structure and defects in photoreceptors of the retina (149).

ALMS1 gen (OMIM 606844), Alström syndrome (OMIM 203800)

Reduced GH reserve indicative of functional GHD has been reported in non-obese patients affected with Alström syndrome, a rare autosomal recessive monogenic disease classified as a ciliopathy disease (150). *Alms1-/-* mice, generated through an ES cell line with gene-trapped *Alms1*, developed obesity, hypogonadism, hyperinsulinemia, retinal dysfunction, and late-onset hearing loss, similar to the human phenotype of the disease (151). Zebrafish embryos depleted of alms1 using morpholinos showed beta-cell decrease in the pancreas (152). This was also validated using a CRISP/cas9 approach (152).

GH Insensitivity ("Primary IGF-I Deficiency")

Insensitivity to GH (GHI) is characterized by low IGF-I levels associated with normal or elevated GH levels and a lack of IGF-I response to GH treatment. Since GH synthesis and secretion are preserved in IGF-I insensitivity, some authors have suggested the term "primary IGF-I deficiency" to differentiate these patients from those with GHD in which IGF-I is low due to the lack of GH ("secondary IGF-I deficiency"). Several genetic defects are responsible for the impairment of GH action resulting in short stature that can affect intrauterine growth or be present in the postnatal period (14,153-155). These disorders involve at least eight different genes (**table 3**).

GHR gene (OMIM 600496), Laron syndrome (OMIM 262500), partial GH insensitivity (OMIM 604271)

Complete GH insensitivity (GHI) was first reported by Laron *et al*. in 1966 (156) in three siblings of Yemenite origin, presenting the classical clinical appearance of GHD but with GH levels that were markedly elevated. Although the possibility of an inactive GH molecule was first hypothesized, the finding that liver membranes prepared from biopsies of these patients were unable to bind iodinated GH, strongly suggested that the alteration resided in the target effector for GH. Cloning of the *GHR* gene opened up the possibility to characterize patients with this condition presenting a partial deletion of the *GHR* gene (157). In 30 patients described by Laron and his colleagues, adult height ranged from 108 to 136 cm (158). Years later, 20 patients with *GHR* deficiency were described among members of an inbred white population from the province of Loja in southern Ecuador (159,160). At least 70 different mutations affecting the *GHR* gene have been reported in more than 300 patients (161). The majority of cases were homozygous for *GHR* gene mutations, usually in consanguineous families (161). In most cases, the mutations affect the extracellular domain of the receptor, resulting in abnormal GH binding and low to undetectable GHBP levels. When the gene defect occurs in the transmembrane or cytoplasmatic domains, GHBP levels could be normal or even high. *GHR* gene mutations may result in defects in receptor dimerization, cell membrane anchorage, or signal transduction (161). Usually, GHI is inherited as an autosomal recessive condition, but a few cases have been reported where heterozygous *GHR* mutations exert a dominant negative effect (162-164). These last cases, as well as those caused by an intronic mutation and the activation of a pseudoexon (165), present less pronounced growth retardation and a milder clinical phenotype. In the most severe clinical cases of complete GHI, rhIGF-I is the only therapeutic option to increase linear growth. However, patients with less severe GHI, such as those presenting heterozygous *GHR* mutations, may benefit from rhGH or from a combination of rhGH and rhIGF-I (164). Ghr-knockout mice showed severe postnatal growth retardation, undetectable GHR and GHBP, and very low levels of IGF-I, all findings similar to what was observed in patients with complete GHR deficiency (Laron syndrome) (25). In addition, this mouse has lower glucose and insulin levels, indicators of increased insulin sensitivity (166). In the liver, lack of GH receptor resulted in a higher abundance of insulin receptor (IR) and increased insulin-stimulated tyrosine phosphorylation of IR, likely mechanisms that could explain the increased insulin sensitivity (167). There is no known zebrafish mutant or morphant for this gene yet.

Table 3. GH insensitivity

Gene OMIM	Phenotype OMIM	Inher.	Human phenotype	Mice phenotype	Zebrafish phenotype
GHR 600946	Laron dwarfism 262500 Partial GH insensitivity 604271	AR AD	Severe growth retardation, high GH and reduced IGF-I levels	Severe postnatal growth retardation, undetectable GHR and GHBP, and very low levels of IGF-I. Low glucose and insulin levels, indicators of increased insulin sensitivity	Unknown
STAT5B 604260	GH insensitivity with immune deficiency 245590	AR AD	Severe growth retardation, high GH, and reduced IGF-I levels. Moderate to severe immunodeficiency. Recurrent pulmonary infections and lymphocytic interstitial pneumonia	Fewer thymocytes and splenocytes and a SCID phenotype	Significant reduction of body weight and size in embryos and adults. Loss of sexual size dimorphism
STAT3 102582	Infantile-onset multisystem autoimmune disease 615952	AD	Variable degree of immune dysregulation and the early appearance of different autoimmune diseases. Partial GH insensitivity	75% perinatal mortality and growth retardation with increased apoptosis in thymocytes	Scoliosis, excessive inflammation and smaller than wildtype. Die at juvenile stages
IKBKB 603258	Immunodeficiency 15 615592	AR	Immune disorder, growth retardation and partial GH and IGF-I insensitivity	Defective induction of HIF-1α target genes including vascular endothelial growth factor. Alteration of innate immunity	Unknown
IL2RG 308380	Severe combined immunodeficiency, X-linked, T-cell-negative, B-cell-positive, NK cell-negative 300400	AR	Severe combined immune deficiency. Some patients present GH insensitivity	Hypoplastic thymuses and a reduced number of lymphocytes. Absence of NK cells	Reduced embryonic lymphopoiesis
PIK3R1 171833	SHORT syndrome 269880	AR	Some patients present low levels of IGF-I with insufficient response to rhGH	Increased insulin sensitivity and hypoglycemia	Angiogenesis defects
IGF1 147440	IGF-I deficiency 608747	AR AD	Growth retardation with deafness and mental retardation	Birth weight of about 60% compared to normal mice. Severe postnatal growth retardation. Increased GH levels	Unknown
IGFALS 601489	Acid-labile subunit deficiency 615961	AR	Severe IGF-I and IGFBP-3 deficiencies with mild growth retardation. Poor response to rhGH treatment	13% smaller at 10 weeks of age and marked reductions of circulating IGF-I and IGBP-3 levels	Unknown

STAT5B gene (OMIM 604260) GH insensitivity with immune deficiency (OMIM 245590)

The family of signal transducers and activators of transcription (STATs) includes seven members that act both as intracellular signaling mediators and transcription factors (168). They are activated by multiple growth factors and cytokines. Although GH activates four members of this family (STAT1, STAT3, STAT5a, and STAT5b), STAT-5b is the key mediator of GH promoting actions. In 2003, a homozygous mutation in STAT5B gene was described in a 16-year-old girl with severe postnatal

growth retardation and IGF-I deficiency (169). She had a history of recurrent pulmonary infections and lymphocytic interstitial pneumonia, presenting immunodeficiency characterized by a defect in T cell immunity. Since STAT5b is also required in the signaling of several cytokines such as interleukin-2 and γ-interferon, it seems likely that the growth failure and the immune defect are both due to its inactivation. Ten patients with STAT5b deficiency have been reported, all presenting severe growth failure, complete GHI and a moderate to severe immunodeficiency. GHI resulted in marked growth retardation which is always present, but with a more variable severity of the immune deficiency and pulmonary disease (170-174). Interestingly, haploinsufficiency for *STAT5B* gene appears to affect growth, since heterozygous carriers are shorter than their wildtype relatives (175). At the time this review was written, heterozygous *STAT5B* gene mutations with dominant-negative effect were described in three families in which affected members presented short stature associated with partial GH insensitivity but not severe immune alterations. These *STAT5B* missense mutations (p.Gln177Pro, p.ALa478Val, and p.Gln474Arg) are robustly phosphorylated upon stimulation but are not able to translocate to the nucleus or to bind STAT5*b* DNA response elements. In addition, these variants are able to dimerize to wildtype STAT5B disrupting the transcriptional function of wiltype STAT5B and exerting a dominant-negative effect (176).

Complete loss of *Stat5* (a and b) in mice leads to Severe Combined Immunodeficiency (SCID). These mice die before or shortly after birth, presenting significantly fewer thymocytes and splenocytes than their wildtype littermates (177). Although there are 2 stat5b genes in zebrafish (*stat5b.1* and *stat5b.2*), it appears that *stat5b.1* is the corresponding homologue of mammalian *STAT5B* in fish (178). Stat5b.1 mutant fish generated using CRISPR/Cas9 results in a significant reduction of body weight and length in both embryos and adult zebrafish (178). Also, sexual size dimorphism was eliminated in these adult fish, where normally females are larger and heavier than males. Interestingly, there seems to be a positive feedback loop whereby stat5b positively regulates *gh1* expression in zebrafish, which is absent in mammals (178).

STAT3 gene (OMIM 102582), infantile-onset multisystem autoimmune disease (OMIM 615952)

Heterozygous gain-of-function mutations in the *STAT3* gene have been reported in patients presenting a variable degree of immune dysregulation and the early appearance of different autoimmune diseases (type-1 diabetes, autoimmune enteropathy, thyroid dysfunction, pulmonary disease, hemolytic anemia, thrombocytopenia, neutropenia, juvenile-onset arthritis, eczema) (179-182). Most of the affected patients present growth failure, normal GH levels, and low IGF-I levels, indicative of some degree of GHI. Constitutive activation of STAT3 is associated with increased expression of SOCS3 (suppressor of cytokines signaling) (179). Members of the SOCS family block STAT activation by turning off the initial signal (183). Epstein-Barr virus-transformed cell lines derived from patients carrying activating STAT3 mutations display reduced STAT5b phosphorylation in response to interleukin-2, a plausible explanation for the observed GHI (181). Patients carrying activating STAT3 mutations preserve some degree of responsiveness to rhGH treatment (181,182). The severity of the immune disorder and autoimmunity caused by germline *STAT3* gain-of-function mutations results in a severe life-threatening condition. Although bone marrow transplantation and anti-IL6R monoclonal antibody have been used as therapeutic tools, the results were not always successful (179,180,182). Potential novel therapeutic approaches include small-molecule inhibitors of STAT3 (181).

Stat3$^{-/-}$ mice die around embryonic day 7 (184). Heterozygous mice carrying the mutation p.Ser727Ala (SA) in one allele and a deletion in the other *Stat3* allele (*STAT3* $^{SA/-}$ mice) have normal amounts of Stat3 in all cells except fibroblasts which have a 25 % or less Stat3 transcriptional response. These mice had 75 % perinatal mortality and growth retardation with increased apoptosis in thymocytes in the surviving mice (185). There are many studies involving the zebrafish *stat3* gene. Morpholinos to knockdown *stat3* have implicated this transcription factor in heart, eye and hair cell regeneration in zebrafish (186-188). Finally, a recent study generated null mutations in zebrafish *stat3* which resulted in mutants that die during juvenile stages exhibiting scoliosis and excessive inflammation (189). They also appeared smaller than wildtype fish. All these zebrafish mutants are models of *STAT3* inactivation, but a gain-of-function *stat3* zebrafish model has not yet been reported.

IKBKB gene (OMIM 603258), Immunodeficiency 15 (OMIM 615592)

The nuclear factor κB family of transcription factors modulates gene expression by binding to specific DNA regulatory elements as homo or heterodimers. In the unstimulated state, NF-κB dimers are bound to IκB preventing translocation to the nucleus (190), thereby maintaining NF-Kb in an inactive state. Heterozygous mutations in IKBKB gene, that encodes for the inhibitory IκBα protein, have been described in two patients with immune disorder, growth retardation and partial GH and IGF-I insensitivity (191).

In the mouse, IKK-β deficiency results in defective induction of HIF-1α target genes including vascular endothelial growth factor (VEGF). IKK-β is an important physiological contributor to the hypoxic response, linking it to innate immunity and inflammation (192). Zebrafish knockdown studies have not been performed in this gene yet.

IL2RG gene (OMIM 308380), Severe combined immunodeficiency, X-linked, T cell-negative, B-cell-positive, NK cell-negative, XSCID (OMIM 300400).

The IL-2 receptor γ common (IL-2Rγc) chain is the shared subunit of the receptors for the IL-2 family of cytokines. IL2RG associates with different interleukin receptor alpha chains to form heterodimers. Through the binding of cytokines, these receptors regulate homeostasis of the immune system. Mutations in the gene encoding the gamma subunit of the interleukin-2 receptor (*IL2RG*) are found in patients presenting X-linked severe combined immunodeficiency (193). Some patients with mutations in the *IL2RG* gene present a diminished or absent response to rhGH treatment both in terms of IGF-I increase as well as growth acceleration (194). In addition, GH stimulation of mutated B cells shows no phosphorylation of STAT5b and lack of nuclear translocation, indicative of a defect in GH signaling (195).

Knockout mice for *Il2rg* gene lack gamma chain expression and have hypoplastic thymuses. Splenic T cells were diminished at 3 weeks of age, and B cells were greatly diminished in contrast to the situation in patients with XSCID (196). There are 2 zebrafish IL-2Rγc paralogs, *il-2ryc.a* and *il-2ryc.b*, and knockdown of *il-2ryc.a* but not *il-2ryc.b* leads to reduced embryonic lymphopoiesis (197).

PIK3R1 gene (OMIM 171833) SHORT syndrome (OMIM 269880)

PIK3R1 codes for the regulatory subunits of the phosphatidyl inositol-3 kinase class IA (PI3K) and is involved in activation of the AKT/mTOR pathway to ensure proper growth and cell proliferation (198). SHORT syndrome historically has been defined by its acronym: short stature (S), hyperextensibility of joints and/or inguinal hernia (H), ocular depression (O), Rieger abnormality (R) and teething delay (T) (199). *PIK3R1* heterozygous mutations have been identified in several patients affected with SHORT syndrome (200-203). Persistently low levels of IGF-I with insufficient response to rhGH has been shown in some patients, indicating some degree of GHI.

Targeted disruption of the *Pik3r1* gene in mice leads to increased insulin sensitivity and hypoglycemia due to increased glucose transport both in muscle and adipocytes (204). In addition, liver-specific deletion of *Pik3r1* in mice also results in increased hepatic and peripheral insulin sensitivity (205). Zebrafish embryos injected with morpholinos to reduce pik3r1 levels displayed angiogenesis defects with variable shortening of intersegmental vessel (ISV) length and were otherwise overtly normal (206).

IGF1 gene (OMIM 147440), growth retardation with deafness and mental retardation due to IGF-I deficiency (OMIM 608747)

The first molecular defect in the IGF1 gene was described in 1996 in a 15-year-old boy presenting severe intrauterine growth retardation, postnatal growth failure, sensorineural deafness, mental retardation, microcephaly, and delayed puberty (207). The patient was homozygous for a deletion of exon 4 and 5 in the *IGF1* gene. Marked insulin-resistance was also present, likely related to the abnormally high GH levels and a functional GH receptor. The few other reported patients with *IGF1* gene mutations present pre- and postnatal growth impairment, mental retardation, and hearing loss (208-211). A homozygous missense mutation (p.Val44Met) detected in a 55-year-old patient presenting severe intrauterine and postnatal growth retardation, microcephaly, and sensorineural deafness was functionally inactive with a 90-fold reduced affinity for the IGF-I receptor (209). The classical phenotype with prenatal growth retardation was observed in those cases with both affected alleles. A less severe phenotype without intrauterine growth retardation, microcephaly, or deafness, has been described in several members of a family, carrier of a frameshift mutation that, if expressed, resulted in a truncated and presumably inactive protein (210). A patient with non-dysmorphic phenotype and less severe pre- and postnatal growth retardation was homozygous for a missense mutation that reduces two- to three-fold the affinity of the mutant IGF-I for the IGF-1 receptor (211). Molecular defects of the *IGF1* gene are rare, and only about 9 patients have been described (212).

In the mouse, targeted disruption of *Igf1* gene resulted in birth weight of about 60% compared to normal mice. Depending on the genetic background, *Igf1(-/-)* dwarf mice die shortly after birth or survive and reach adulthood (213). To further explore the role of liver-produced IGF-I, the major contributor of circulating IGF-I, Yakar *et al* (28) used the Cre/loxP recombination system to delete the *igf1* gene exclusively in the liver (LID mouse). Although the LID mouse showed a severe reduction in circulating IGF-I levels, body weight, body length, and femoral length did not differ from wildtype littermates. However, due to the reduction of negative feed-back, this animal presents high circulating GH levels that could partially compensate for the reduction of circulating IGF-I levels. This study suggests an important role for locally produced IGF-I (autocrine/paracrine IGF-I) in longitudinal growth. The only study that used morpholinos to knockdown *igf1* in zebrafish led to embryonic abnormalities that were not possible to discern from nonspecific toxic effects from the morpholino itself (214).

IGFALS gene (OMIM 601489), acid-labile subunit deficiency (OMIM 615961)

The acid-labile subunit (ALS), a member of the leucine-rich repeats proteins, is a circulating protein that plays an important role in maintaining high circulating levels of IGF-I. Although ALS has no discernible affinity for IGF-I, it is capable of binding binary complexes formed by IGF-I or IGF-II with IGFBP-3 or IGFBP-5, forming ternary complexes (215).

Thus, ALS could be considered a binding protein of binary complexes. The main role of ALS is to maintain up to 80-90% of the circulating IGFs in this ternary complex, extending the half-life of free IGF-I from 10 min to more than 12 hours (216).

The first description of complete ALS deficiency was reported in a 17-year-old boy with delayed onset of puberty, slow pubertal progress, and markedly reduced IGF-I and IGFBP-3 levels that remained unchanged after GH stimulation (217). The patient was homozygous for a frameshift mutation in the IGFALS gene (p.Glu35Lysfs*87). Once the clinical characteristics and biochemical phenotype of ALS deficiency became recognized, severe IGF-I and IGFBP-3 deficiencies associated with moderate growth retardation (a "mismatch" between the severity of IGF-I and IGFBP-3 deficiencies and the mild effect on growth), several reports communicated at least 62 patients with this defect (218-228). In these patients, whereas circulating levels of IGF-I are dramatically decreased, local production appears to be preserved. Local expression of IGF-I, under the control of normal and/or increased GH levels, could be responsible for the preservation of linear growth near normal limits (229-231). Functional *in vitro* characterization of several *IGFALS* variants has shown that pathogenic variants result in the absence of ALS synthesis or intracellular retention of the mutant protein (232,233). In children with apparent GHI, systematic genetic characterization by candidate gene approach or WES has shown that mutations in the IGFALS gene, resulting in complete ALS deficiency, is the second most frequent gene defect, second only to *GHR* gene defects (234).

It is noteworthy that heterozygous IGFALS gene mutations are present in a subgroup of children with idiopathic short stature presenting partial ALS deficiency (235,236). Characterization of children with partial ALS deficiency may prove clinically relevant, because these patients have shown responsiveness to rhGH treatment, increasing IGF-I levels and accelerating growth velocity (236,237). Whether this initial response results in an increase in adult height remains to be determined.

Homozygous null mice for *Igfals* are 13% smaller than their wildtype littermates at 10 weeks of age (238). This modest phenotype, despite marked reductions of IGF-I and IGFBP-3 levels in plasma, support the importance of locally produced IGF-I in growth.

The zebrafish *igfals* morphants have not yet been reported.

IGF-I Insensitivity

There are only a few molecular defects resulting in impairment of IGF-I action (**table 4**).

IGF1R gene (OMIM 147370), insulin-like growth factor I, resistance to (OMIM 270450)

A specific IGF-I receptor was first characterized in 1977 (239), but it was not until 1986 that the complete cDNA sequence for this receptor was published (240). Although several patients with intrauterine growth retardation presenting elevated levels of GH and IGF-I, suggestive of some degree of IGF-I resistance, were reported in the 1980s and 1990s, (241-243), it was not until 2003 that the first patients with *IGF1R* gene mutations were reported (244). The first mutations in this gene were detected in patients with intrauterine growth retardation or short stature and elevated IGF-I levels (244). This original report was essentially the result of two separate

Table 4. IGF-I resistance

Gene OMIM	Phenotype OMIM	Inher.	Human phenotype	Mice phenotype	Zebrafish phenotype
IGF1R 147370	Resistance to IGF-I 270450	AR AD	Intrauterine growth retardation, postnatal growth retardation with normal/ elevated IGF-I levels	Mice are 45% smaller than widtype at birth with general organ hypoplasia	Reduced embryonic growth, arrested development, and increased lethality. Defects in retina, inner ear, heart, and motor neurons
PAPPA2 -	PAPPA2 deficiency -	AR	Mild postnatal growth retardation with high levels of IGF-I, IGFBP-3, and ALS	Postnatal growth retardation	Ventral curvature of embryos, notochord defects, reduced jaw and angiogenesis defects
IGF2 147470	Severe growth restriction with distinctive facies 616489	Epigenetic	Severe intrauterine and postnatal growth restriction and a Silver-Russell syndrome-like phenotype	Heterozygous males have growth defects while females are normal. Homozygous males and females have growth defects	Ventralized embryos with reduced growth, reduced eyes, disrupted brain structures and a defective cardiovascular system

studies published together. The first group consisted of 42 patients with intrauterine growth retardation and subsequent short stature. One girl was a compound heterozygote for a point mutation in exon 2 of the *IGF1R* gene (p.Arg108Gln/p.Lys115Asn). Cultured fibroblasts from the patient had decreased IGF-I-receptor function. In the second cohort of 50 children with short stature and elevated circulating IGF-I levels, the authors identified one boy with a nonsense mutation (p.Arg59*) that resulted in a reduced number of IGF-I receptors in fibroblasts. Both children had intrauterine growth retardation and poor postnatal growth. It is likely that, as is observed in the mouse, complete absence of IGF1R in humans may be lethal. This could explain why, except for two compound heterozygous cases (244,245), and two homozygous patients (246,247), only heterozygous cases have been reported. The few patients presenting mutations in both *IGF1R* alleles appear to retain some degree of IGF1R activity. Functional *in vitro* studies of naturally occurring *IGF1R* mutations suggest that different mechanisms could explain the impairment of IGF action: receptor haploinsufficiency, decreased biosynthesis, reduced binding affinity, interference of transmembrane signaling, and disruption of the tyrosine kinase activity (248). The impact of *IGF1R* mutations on intrauterine growth is variable, but is frequently more severe when maternally inherited, indicating that maternal IGF-I resistance during pregnancy is one factor contributing to the severity of growth retardation, possibly by decreasing placental growth (249). As many as 20 patients have been described with *IGF1R* mutations (250-256). These patients have shown a poor to moderate clinical response to rhGH treatment (248).

Targeted disruption of the *Igf1r* in mice led to death shortly after birth due to respiratory failure and 45% smaller birth size than wildtype mice. They also have global organ hypoplasia (213). Zebrafish have 2 *igf1r* genes (*igf1ra* and *igf1rb*). Using either morpholinos or a dominant negative igf1r fusion protein to target these 2 genes in zebrafish resulted in reduced embryonic growth, arrested development, and increased lethality. In addition, these embryos had defects in the retina, inner ear, heart, and motor neurons (257).

Pregnancy-associated plasma protein A2 deficiency (PAPP-A2)

A completely new syndrome has been recently described, involving the first genetic defect in a protease. Pregnancy-associated plasma protein-A2 (PAPP-A2) is a serum and tissue protease responsible for proteolysis of IGFBP-3 and IGFBP-5, regulating the bioavailability of IGF-I and IGF-II to their target tissues (258). Five affected subjects from two families presenting moderate growth retardation and elevated circulating levels of IGF-I, IGF-II, IGFBP-3, IGFBP-5, and ALS, were found to be homozygous for two different mutations in the *PAPPA2* gene (p.Asp643fs25* and p.Ala1033Val) (259). *In vitro* analysis of IGFBP cleavage demonstrated that both mutations cause a complete absence of PAPP-A2 proteolytic activity. Size exclusion chromatography showed a significant increase in IGF-I bound in its ternary complex, and decrease in free and bioactive IGF-I concentrations. Other clinical findings included characteristic thin long bones, most notable in the fibulae, tibiae, and femurs. While bone age was according to chronological age, bone mineral density (BMD) was decreased at the lumbar spine, and fasting glucose concentrations were normal with mild hyperinsulinemia. Interestingly, a one-year treatment with rhIGF-I resulted in a clear increase in growth velocity and height in two siblings. Bioactive IGF-I was increased, and spontaneous GH secretion was diminished after acute administration of rhIGF-1, whereas serum total IGF-I and IGFBP-3 levels remained elevated (260). Besides the moderate increase in growth velocity, one-year treatment with rhIGF-I resulted in a reduction of insulin resistance and an increase in total body BMD (261).

The finding of *PAPPA2* mutations as an etiological cause of short stature has both clinical and physiological consequences. The molecular diagnosis resulted useful for the selection of the proper therapeutic agent to increase adult height and, on the other hand, illustrate the important physiological role of the IGFBPs and their specific proteases in the regulation of IGF-I bioavailability (262).

The *Pappa2a* knockout mouse was of normal size at birth but had postnatal growth retardation (263). The knockout mice also display disproportionally reduced dimensions of specific bones, including skull and mandible (264). Knockdown of zebrafish *papp-a2* results in ventral curvature of embryos as well as notochord defects implicating this protein in notochord development. In addition, the jaw is significantly reduced, indicating a role for *papp-a2* in cranial cartilage development. Finally, embryos also have defects in angiogenesis (265).

IGF2 gene (OMIM 147470), severe growth restriction with distinctive facies (OMIM 616489)

In 2015 Begemann *et al*. reported an *IGF2* nonsense variant (p.Ser64*) in a multigenerational family with four members presenting growth restriction (266). Only transmission of the paternally affected allele resulted in growth impairment in those tissues involved in growth, confirming the monoallelic expression of the maternally imprinted *IGF2* gene. The affected patients have severe intrauterine and postnatal growth restriction and a Silver-Russell syndrome (SRS)-like phenotype. More recently, two independent reports described two patients with a frameshift (p.Leu37Glnfs*31) and a missense (p.Gly34Asp) de novo mutation in the *IGF2* gene, presenting a characteristic SRS-phenotype, (267,268), indicating that this alteration could arise as a *de novo* condition in non-familial patients affected with SRS. Remarkably, in these last cases the mutations resided in the

paternal allele, an expected result considering the paternal monoallelic expression of the IGF2 gene in non-hepatic tissues. Two out of three patients showed a satisfactory growth increase after chronic rhGH treatment (267).

Targeted disruption of *Igf2* in mice led to heterozygous male mice with growth defects yet phenotypically normal heterozygous females. Homozygous female mutants resemble their heterozygous growth defective male siblings (269). In contrast, the zebrafish genome contains 2 co-orthologs of mammalian *IGF2* gene (*igf2a* and *igf2b*). Knockdown of either gene using morpholinos led to ventralized embryos characterized by reduced growth, reduced eyes, disrupted brain structures and a defective cardiovascular system. Knockdown of both genes simultaneously increased the severity of the phenotype. This implicates both genes in dorso-ventral patterning during development in zebrafish (270,271).

Conclusions

From the molecular characterization of the first genetic defect in the GH/IGF axis, a complete GH1 gene deletion in patients with severe isolated GHD and profound growth retardation by Phillips III and their colleagues in 1981, mutations in more than 48 different genes have been described all along the GH/IGF axis. These defects result in alteration of GH synthesis/secretion (isolated or associated with other pituitary hormones), defects in GH action (alteration at the level of the GH receptor, the intracellular signaling pathway, or the transport of IGFs), or at the level of IGF-I (alteration of IGF-I synthesis transport or action). Most of these molecular defects were discovered by the candidate gene approach, by using clinical data and biochemical profiles to select the more likely candidate gene(s) to be studied. Since 2012, with the development of next generation sequencing (NGS) techniques, capable of determining the WES or even the whole genome sequence (WGS) within weeks, new genetic clinical conditions have been elucidated in patients where clinical and biochemical data did not suggest an obvious candidate gene or where several likely candidate genes had to be explored and the conventional sequencing of each one would be more expensive and time consuming than the NGS approach. In addition, this last approach has revealed novel genetic defects, previously unknown or unsuspected given the clinical and biochemical characteristics of the subjects under study. It has also been shown that in a small percentage of cases, more than one gene could be affected, resulting in a more complex clinical presentation, usually presenting overlapping phenotypic features (272). It has been proposed that genetic evaluation of short stature is indicated in those cases that present severe GHD, multiple pituitary hormone deficiency, unequivocal GH insensitivity, small for gestational age without catch-up growth, additional congenital anomalies or dysmorphic features, evidence of skeletal dysplasia, associated intellectual disability, microcephaly, and severe growth retardation (273). Even with careful selection of patients, a genetic diagnosis is obtained in only 30-40% of patients with IGHD, CPHD, apparent GH or IGF-I insensitivity (13,234). Due to increased accessibility to NGS, a significant number of gene variants have been described all along the GH/IGF axis. These variants appear both in genes previously associated with these conditions as well as in completely novel genes. Characterization of these variants by functional *in vitro* assays and *in vivo* animal models is required to determine the real contribution of these findings.

Disclosure

The authors do not have any commercial or financial relationships that could be considered as a potential conflict of interest.

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Pediatric Growth Hormone Deficiency (GHD) in the Recombinant Human GH (rhGH) Era

Michael B Ranke

Prof emeritus, Children´s Hospital, University of Tübingen, Germany

Corresponding author: Michael B. Ranke, MD, PhD, FRCP (Edin), Prof. emer, University Children's Hospital, Hoppe-Seyler-Str. 1, 72076 Tübingen, Germany, Tel: +49 7071 76805, Fax: +49 7071 294157, E-mail: michael.ranke@gmx.de

Abstract

During the phase of using hGH extracted from pituitaries (pit hGH) - 1958-1985 - fundamental experience related to the diagnosis and treatment was accumulated. However, since recombinant hGH (rhGH) had become available diagnosis and treatment of GHD were conducted world-wide in a more standardized way. Treatment with rhGH was also accompanied by documentations in large international pharmaco-epidemiological surveys, which provided new insight. Despite of this development the treatment of children and adolescents with GHD raises still issues related to the most effective and efficacious as well as safe use of rhGH. This brief review attempts to discuss a few aspects related to these topics as they have developed during the rhGH era.

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Introduction

The time periods of treatment with GH are commonly divided into the "pituitary hGH era" starting in 1958 and ending in 1985 with the CJD disaster (see chapter by Laron) and the "recombinant hGH era" definitely beginning with the approval of methionyl rhGH in1985. Both eras were – and are - accompanied with major methodological developments and discoveries which allowed the broadening of our understanding of the GH system, its deficiencies and subsequently the treatment of these (1). During the pituitary GH era the major regulatory mechanisms of pituitary GH secretion, the basic genetics of pituitary hormone deficiencies and the IGF-IGFBP system had already been discovered. The various metabolic effects of GH and their therapeutic potential had also been conceived. However, due to methodological limitations and restrictions regarding the availability of pit-hGH this knowledge had found only limited access into clinical research or even the practice of clinical paediatric care. The almost equally long era of rhGH was accompanied with an enormous wealth of new developments such as in medical standardisation, in hormone measurements, in molecular genetics and in body imaging techniques - all embedded into the new IT technology. In contrast to the years before the introduction of rhGH, new technical developments have become accessible to practicing physicians almost immediately after their discovery. In addition, the understanding of paediatric GHD was influenced by the recognition of adult GHD. It is the aim of this review to focus on a few aspects related to the developments of the diagnosis, treatment and follow-up in GHD during the recombinant GH era.

Classification of GHD

GHD has traditionally been classified into various categories without following a clear and uniform principle such as: congenital/non-acquired vs. acquired; isolated vs. combined

(with other pituitary deficits); complete vs. partial; causal vs. idiopathic; permanent vs. transient. The new classification systems developed during the rhGH era have attempted to classify GHD more systematically according to their causes, frequently based on molecular genetics and neuroimaging (2-5). However, it needs to be kept in mind that a disease classification is a step after the principal diagnosis GHD has been ascertained anthropometrically and biochemically. However, the problem remains (as in many other hormonal deficiencies) that GHD mostly presents as an impairment, not as a complete absence and that it mostly occurs without evidence for a cause and without additional supporting findings, thus termed "idiopathic".

Diagnosis of Childhood GHD

Auxological Parameters

There are numerous review articles about how to diagnose and treat GHD in children without reaching uniformity (6-11). All of these outline that the suspicion of GHD rests on anamnestic, clinical and anthropometric criteria but that the diagnostic proof is gained from biochemical criteria. Anthropometrical criteria such as short stature and slow growth are commonly shared by a variety of disorders, and there is no single one parameters serving as proof of an impaired GH secretion. Textbooks define the typical growth disorder in childhood caused by GHD as severe and proportionate, with a relatively large neuro-cranium and acromicria, which gives affected children a doll-like appearance. Such a verbal description of GHD needs to be filled with numerical evidence gained from measurements such as height, weight and other auxological parameters and their comparison with appropriate normative data– potentially longitudinally. In prepubertal children with non-acquired GHD, body height – the commonly presenting sign - should be at least 2.0 SDS (or the 3rd centile) below average for age and should be approximately 1.5 SD below the familial height target (12). Height velocity measured over at least 6 months should be below the 25th centile for age. A normal bone age makes GHD unlikely. The typical reduction in muscle mass and the enhancement of fat mass can be verified with a variety of methods (anthropometry, BIA, DXA, CT, MRI) most of them standardized and currently easily available. Thus, in the pre-rhGH era with the then mostly severe cases presenting it was relatively easy to suspect GHD on clinical grounds only, while in the less severe it is required to determine the likelihood of GHD by balancing a multitude of findings.

The biochemical evaluation to verify GHD should commonly be started after the exclusion of other potential causes if the anthropometrical data are suggestive of GHD and/or if there are strong indicators of an acquired or congenital intracranial lesion, signs of multiple pituitary hormone deficiencies or neonatal symptoms and signs of GHD. In order to avoid misclassifications and unnecessary testing it needs to be considered that parameters of growth are difficult to interpret specifically in early childhood and during peripubertal age (13).

Growth Hormone Measurement

The first radio-immunoassay for hGH was described in 1963 (14). Since then a variety of immunoassay techniques are available for clinical routine, and exact measurements by mass spectroscopy (15,16) may be used in the future. GH measurements can today also be made in minute amounts of blood (17). One main aspect for the correct diagnosing of GHD is the standardization of GH measurements (15). Part of this is the availability and use of international reference preparations (IRPs) which have changed over time. The first IRP for hGH [IRP 66/217 (1969)] was of pituitary origin with a designated specific activity of 2 IU/mg. Subsequent IRPs [e.g., IS 98/574] were pure 22 kDa recombinant hGH with an assigned potency of 2.6 and 3 IU/mg, respectively. Thus 20 µIU hGH before 1982 were equivalent to 10 ng, but are currently only 6.7 ng (18). In addition, while in the pit-hGH era polyclonal antibodies directed against multiple epitopes of hGH were used, subsequently more often monoclonal antibodies directed against different epitopes of monomeric 22 kDa rhGH were applied.

Quantifying GH Secretion

Since GH levels measured in a random blood sample does not reflect the GH secretory status of an individual a quantification of GH secretion needs to be attempted by GH measurements in samples drawn frequently over time or after the provocation of GH secretion. Soon after GH could be measured in blood its pulsatile nature of secretion was discovered. The complex mechanisms of its regulation were described in the 1990ies and are still part of ongoing research. The quantification of spontaneous GH secretion over time (12-24 h) is based on measurements in frequently sampled blood specimen combined with calculation algorithms of with the deconvolution technique has gained wide use (19,20). In pediatrics the quantification under physiological and pathological conditions has been evaluated extensively (21) and normative data have been developed. Since it is cumbersome and expensive to do long-term frequent sampling in a clinical setting this highly valuable and physiological approach is not widely used. It is however essentially required to diagnose "neurosecretory dysfunction" of GH secretion (NSD) a special case of idiopathic GHD which was "fashionable" during the early years of rhGH (22).

Multiple diagnostic procedures (GH stimulation tests) were developed provoking GH secretion. It is assumed that the GH levels – or even only the maximum level used as a cut-off - during such procedure can discriminate GHD from normal. Many test procedures provoking GH secretion applying various

agents (e.g. arginine, glucagon, propranolone, L-Dopa) and their combinations have been published (23,24).

The first of these stimulation tests - considered the golden standard for a long time - was insulin-induced hypoglycemia (ITT). None of the 53 children with clinically evident GHD investigated by Roth *et al*. in 1963 exceeded the level of 5 ng/ml (25), while all 37 non-GHD children investigated by Frazier in 1967 exceeded a concentration of 5 ng/ml (26. Thus, in the late 1960ies 5 ng/ml (then equivalent to 10 µU/ml) 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 (=3.5 ng/ml) was not surpassed in one test, or 7- 15 mU/L (= 3.5-7.5 ng/ml) was not surpassed in two standard tests. After 1990 most physicians, rather arbitrarily, accepted a maximum level of 10 ng/ml as the cut-off, which potentially led to an excess of patients diagnosed with idiopathic isolated GHD.

In addition, since physiologically GH secretion is relatively low just before puberty onset, the heavily disputed "priming" with sex steroids before performing GH provocation tests has recently been advocated to avoid false positive results in suspected cases during peripubertal age (11). Only in recent years evidence re-emerged that considering modern methods and references the cut-off should be near 7 ng/ml (27).

The varying mechanisms by which test agents act at the pituitary or hypothalamic level (partly including GHRH), their potential risks, the variability of response, the lack of reproducibility and the paucity of truly normative data and – not least - the principle of the statistical variance of any biological parameter - has made a cut-off driven diagnostic approach highly disputed. More so, any laboratory parameter is only of diagnostic relevance if it is in accordance with the overall evidence.

IGF-Parameters

IGF-I, IGFBP-3 and ALS which all together form a "ternary complex" in the circulation are GH dependent. However, the production of each of the "IGF-proteins" is also dependent on other factors (e.g. nutrition, age, sex steroids) and the levels of these parameters in blood/tissues is not only dependent on their production but also on their degradation. Thus, low blood levels of IGF-I may be the result of low GH secretion (a case of "secondary" IGF-I deficiency) (3), or of low "primary" IGF-I generation, or IGFBP-3 or ALS deficiencies. The exact relationship between statural growth, GH levels and the levels of IGF-parameters (specifically IGF-I and IGFBP-3) in blood is still not clear.

During most of the rhGH era, immunoassays of IGF-I and IGFBP-3 have been available and have been progressively standardized (15,28,29). Since their blood levels are subjected to little daily variation it was assumed that a single blood level could accurately reflect GH secretion, thus potentially making elaborate GH test unnecessary. Aside from methodological issues (assays, references) the reports about the diagnostic value of measuring IGF parameters were biased by the a priory correct/incorrect classification into GHD or non-GHD of the short children investigated. Since the classification depended on anthropometric and GH cut-off levels these investigations gave incoherent results, but overall agreed that the diagnostic specificity of IGF-I (IGFBP-3) measurements is higher than its sensitivity. Today clinicians would agree that "normal" levels of IGF-I (e.g. IGF-I > -1.0 SDS) or IGFBP-3 (e.g. IGFBP-3 > 0.0 SDS) are not likely in children with true GHD, and are often the reason for not conducting further GH testing in short children.

Combined GHD, structural anomalies of the pituitary and gene defects

The combination of GHD with other pituitary hormone deficiencies had been suspected in affected children on clinical grounds (e.g. icterus prolongatus, microphallus, absent puberty) and could be proven biochemically already during most of the pit hGH era. Despite of the refinement in diagnostic tools the diagnosis of gonadotropin deficiencies before pubertal age is still difficult today.

During the pit hGH era it had been realized that a number of syndromes associated with facial malformations (e.g. holoprosencephaly, empty sella syndrome, septo-optic dysplasia) were associated with GHD and other pituitary deficiencies and could be associated with complex syndromes (e.g. Rieger syndrome) (30). It was also recognized that various forms of isolated GHD occurred with different modes of inheritance (31). With the advent of molecular genetics the molecular basis for a great number of developmental disorders of the hypothalamo-pituitary region, of the pituitary cell lineage and of the GH gene were discovered (5,32-34).

The magnetic resonance imaging (MRI) technique opened the path to the non-invasive imaging of the pituitary region allowing to describing the region in terms of structure and size. Today MRI investigations of the cranium are standard in children with suspected GHD and should probably be performed in every child treated with GH for GHD. Anatomical abnormalities are often observed in children with suspected idiopathic GHD (36-38). However, while the finding of a gene defect in a child with presumed impairment of GH secretion provides very strong evidence for the assumption of the existence of "true" GHD, this is less so with regard to some findings made by MRI. Structural anomalies of the pituitary region such as the combination of a hypoplastic anterior pituitary plus missing/interrupted stalk plus ectopic posterior pituitary (HME) provide strong evidence for severe and mostly combined GHD, while a subnormal size of an eutopic anterior pituitary alone provides less strong evidence for the existence of GHD.

Prevalence – Incidence

Any form of GHD - idiopathic or acquired, combined or isolated – is rare and the diagnostic criteria used in reports vary. Therefore it is difficult to obtain solid epidemiological data about GHD in childhood. In the pit hGH era when very short (height < -3 SDS) children were investigated and a GH cut-off of 4.5 ng/ml was applied, the prevalence was estimated to be 1: 4.000 (39). During this period applying a GH cut-off of 5 ng/ml the prevalence was estimated to be in the range of 1 to 5.000-30.000 (40). During the rhGH era applying a GH cut-off of 10 ng/ml an incidence of 1: 3.400 was reported (41). The prevalence applying a GH cut off of 10 ng/ml was reported to be 1: 29.000 (42). All these figures can only serve as rough estimates. There has always been a higher prevalence of males diagnosed with GHD (43) and it still remains obscure whether this is biological or due to social biases. Notably in the study from the National Danish registry the incidence of childhood-onset (CO) GHD almost doubled in boys and girls from 1987 on, that is shortly after rhGH had been approved for treatment (44).

GHD during transition

During the rhGH era it has been recognized that GHD in adults– either acquired during adult life (adult onset = AO) or already existing during childhood (childhood onset = CO) - is also a treatable disease (45). It has also been established that in persistent CO GHD the replacement with GH needs to be continued beyond the achievement of adult height in order to ensure the development of normal adult body composition and a normal metabolism between adolescence and adult life, called transition period (46). This concept made it necessary to establish procedures and criteria for the verification of permanent GHD of the children diagnosed and treated during childhood during transition. Without attempting reviewing the vast literature about the results of testing CO GHD children in transition it can be concluded that less than at least 50% of the individuals (mostly with isolated idiopathic GHD) tested during transition do not qualify for the diagnosis of "permanent" GHD. It is assumed that those not qualifying had no proper GHD in the first place, not least because of applying a too high GH cut-off to tests. However, the notion that "transient" GHD in childhood is an existing entity should not completely be dismissed (47).

Treatment of GHD

Aims of GH Treatment

The aims of GH replacement in GHD are focused on efficacy aspects, on safety and efficiency. Efficacy in children was and is primarily focused on growth: rapid catch-up, normal maintenance, appropriate timing and magnitude of pubertal growth, and adult height within the familial target range. In recognition of adult GHD efficacy in children with GHD should also consider the achievement of a normal body composition and functioning as well as the normalization of biochemical abnormalities associated with GHD.

Mode of Application and Dosing

During the pit hGH era injecting GH 2-3 times per week by the i.m. route was common (usually the full amount of an ampule was injected). In the rhGH era it became standard to inject the weekly rhGH dose by dividing it into daily injections via the s.c. route. The latter was assumed to better mimic the physiological pattern of GH secretion and indeed showed to give a higher growth response per GH dose (48). This rather painless mode of injection - often with easy-to-handle devices called pens - also allowed injections to be given by parents and/or patients themselves, thus also opening the door to greater non-adherence.

Only during the rhGH era the appropriate replacement dose in GHD was derived by means of multiple sampling techniques and deconvolution analysis which allowed to determine the secretion rates of GH in prepubertal and pubertal children (19,21). In prepubertal children this averaged to about 0.02 mg (0.06 IU)/kg body weight per day. The doses approved by medical authorities correspond to these finding. However, the replacement doses observed vary world-wide and tend to be below the maximally approved doses (49). While most physicians dose GH according to body weight (kg), some physicians prefer to dose according to calculated body surface area (m^2). During the first year of GH replacement the response in terms of height velocity was found to be positively related to the logarithm of the dose given (50). An increase of GH doses during puberty, though meaningful in some individuals, has overall shown to be neither very effective nor efficacious (51,52).

Adult Height Reached

A number of reports about adult height of children with GHD – males or females, isolated GHD or combined pituitary hormone deficiency – from the two periods of GH availability have been summarised by Guyda in 1999 (53). Subsequently the results from two large observational studies using rhGH (KIGS, NCGS) have been reported (54-57). Of the factors affecting adult height outcomes in terms of gains in height, the age at onset (the earlier, the better) and the responsiveness to GH as observed during the first year (the more, the better) are major components (58). Within the frame of the GH doses given there was no major effect of GH dosing observed. However, this may need further evaluation. In general, compared to the pre-rhGH era during the rhGH era children were less severely GH deficient, predominantly had an isolated GHD and were younger and less short at treatment start. While the gain over initial height was smaller a greater proportion of these

children reached a normal height or a height within their familial target (**table 1**). Even today it must be stated that the goal of height normalization is not reached in all non-acquired GH deficient children.

Optimizing and Individualizing GH Treatment in GHD

During the rhGH era the ideas has been developed to individualize and optimize GH treatment. This has led to the concepts of either designing treatment according to the individual responsiveness to treatment determined with the help of growth prediction models (58,59) or treating according to the IGF-I levels observed (60). The latter concept is practised in adults with GHD. While growth prediction models are well investigated and are partly easily accessible (59,61) the model based treatment approach is still in its beginnings.

GH safety will be discussed in another article in this issue.

Conclusions

With regard to the diagnostic criteria it can be concluded that in the rhGH era anthropometrical criteria have been defined and are accepted which justify biochemical investigations for the verification of the GHD diagnosis. In children anthropometrical investigations with new technologies and the investigation of metabolic parameters, which are common during the process of diagnosing GHD in adults, are still not a part of the diagnostic repertoire. The diagnostic tools - biochemical and other - have been generally available and are more standardized today. The increase of the frequency of – mostly idiopathic isolated – GHD during the first two decades of the rhGH era is foremost the result of the then accepted GH cut-off level (10 ng/ml) to standard tests. Today treatment of GHD follows guidelines developed by experts based on evidence. This and the optimization and individualization of GH replacement offer the chance of achieving a normal height in practically all children with non-acquired GHD. The future will show whether long-acting rhGH will open a new era in the treatment of GHD (62).

Disclosure

The author has no interfering interest to disclose.

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Table 1: Height at start and at end of GH. Pre rhGH and rhGH era: *Representative reports*

Study (§)	Gender	N	Age (yrs)	Ht (SDS)	Ht (SDS)	dHT (SDS)	Years GH (yrs)
				Start GH	FH	Start to FH	
Guyda (53) $ pre-rhGH*	m/f	792	?	-4.2	-2.3	1.9	5.5
Guyda (53) $ rhGH	m/f	4.529	?	-2.9	-1.4	1.5	6.2
KIGS (55,57)	m	792	9.8	-2.9	-1.0	1.7	8.1
	f	466	8.7	-3.2	-1.3	1.6	7.9
NCGS (54-57)	m	2.095	11.3	-3.0	-1.1	1.4	5.2
	f	1.116	10.1	-3.1	-1.3	1.6	5.0

§ *IGHD/MPHD*

$ *summarizing various reports*

******* *partly organic*

FH *Final Height reported*

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Adult Growth Hormone Deficiency: from Transition to Senescence

Jens OL Jørgensen[1], MD, DMSci, Kasper Hermansen[1], MD, Kirstine Stochholm[1], MD, PhD, DMSci, Anders Juul[2], MD, PhD, DMSci

[1]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, [2]Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Correspondence author: Jens OL Jorgensen, MD, DMSci, Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark, E-mail: joj@clin.au.dk

Abstract

The acute metabolic actions of hGH were discovered in GH-deficient adults (GHDA) 60 years ago and placebo controlled trials of prolonged rhGH replacement therapy appeared 30 years after. Untreated GHDA causes excess morbidity and mortality from cardiovascular disease and the clinical features include fatigue, reduced aerobic exercise capacity, abdominal obesity, reduced lean body mass, osteopenia, and elevated levels of circulating cardiovascular risk biomarkers. Several of these abnormalities normalize with GH replacement. Frequent side effects are fluid retention and insulin resistance, which are reversible and dose-dependent. The dose requirement declines with age and is higher in women. Continuation of GH replacement into adulthood is indicated in some patients with childhood-onset disease so the diagnosis must be reassessed. Observational data show that mortality in GH replaced patients is reduced compared to untreated patients. Thus, GH replacement in GHDA has proven beneficial and safe.

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Key words: *Growth hormone, Growth hormone deficiency, Growth hormone replacement therapy, Hypopituitarism*

Introduction

Anabolic activity of pituitary extracts was discovered almost a hundred years ago and GH was isolated in 1944 and tested in human subjects by several groups (1). The protein anabolic and lipolytic effects of hGH were thoroughly investigated by Maurice Raben (2,3) who was the first to suggest GH replacement therapy in adults (3). GH from human cadaveric pituitaries was used to promote longitudinal growth in children with hypopituitarism, but the limited supply impeded exploration of other indications. The use of pituitary hGH stopped in many countries in 1985 since it was associated with transmission of Creutzfeld-Jakob disease (4), which accelerated the approval of rhGH (5). The potentially unlimited supply of rhGH enabled new indications to be studied, the first of which was GH replacement in adult patients with GH-deficiency (GHD)(6,7). This review provides a brief history and update of this therapeutic field.

The Early Studies

The first placebo-controlled trial was performed as an investigator-initiated collaboration between adult and pediatric endocrinologists and published in 1989 (6). The

patients had childhood-onset GHD reconfirmed by a GH stimulation tests and had received rhGH replacement for a mean period of ≈ 7 years, which was stopped ≈ 6 years prior to the study. The mean age at study start was ≈ 24 years. The study had a crossover design with 4 months treatment periods separated by a 4 months washout period. The daily rhGH dose was 4 IU/m^2 body surface (≈1 mg/day). The major outcomes were muscle and fat volume of the thigh assessed by CT scan, isometric muscle strength, and aerobic exercise capacity assessed on a bicycle ergometer. In addition, glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured isotopically. A significant increase in muscle volume and a significant reduction in fat volume were recorded, which was accompanied by a significant increase in exercise capacity. Both GFR and RPF increased significantly and became normalized. As expected, a significant treatment-induced increase in serum IGF-I levels occurred. Additional publications from this trial revealed GH replacement to induce marked and concerted elevations in the levels of biomarkers of bone remodeling (8,9). It was also demonstrated that rhGH stimulated the extrathyroidal conversion of T4 to T3 (10), and restored normal sweating capacity (11). The original study was continued by an open phase of rhGH therapy, which documented continued improvement in body composition, exercise capacity, muscle strength, and forearm bone mineral content (12,13).

The second placebo-controlled trial (7) mainly comprised patients with adult-onset GHD due to a pituitary tumor and its treatment. It had a parallel design with a 6 months treatment period where each patient was examined before and after. The daily rhGH dose was ≈ 2 mg and the mean age of the patients was ≈ 39 years. The main outcomes were body composition assessed by conventional anthropometry and total body potassium, and resting energy expenditure using indirect calorimetry. Lean body mass (LBM) increased significantly together with a significant reduction in fat mass. This was associated with an increase in resting energy expenditure also after correction for LBM. In addition, evidence of GH-induced insulin resistance was recorded. Beneficial effects of GH replacement on exercise capacity and hyperlipidemia were also reported from the original study (14-16).

The Syndrome of GH-Deficiency in Adults

The term 'syndrome' was adopted to describe the emerging clinical picture of GH-deficiency in adults (GHDA) and the effects and side effects of GH replacement. (17) This concept was substantiated by studies of the clinical features of the treatment naïve patient (18-20) and the results of numerous new treatment trials (**figure 1**) (21-24).

GH-untreated patients with hypopituitarism have an increased overall mortality (25-29). The annual incidence of GHDA is approximately 15 per million and higher in men (30) and the estimated prevalence is approximately 250 per million. The syndrome overlaps with the metabolic ditto as regards visceral obesity, hyperlipidemia, and atherosclerosis. Moreover, premature cardiovascular morbidity and mortality is present in GH-untreated hypopituitary adults (25,31), whereas echocardiographic studies document positive inotropic effects of GH replacement (32,33-35). It was also observed that untreated GHDA is accompanied by reduced total body water and extracellular fluid volume (18). This reverses by GH replacement (36,37) to the extent that over hydration is the most frequent and dose-dependent side effect of GH replacement. The mechanisms involve sodium retention mediated by activation of the renin angiotensin aldosterone system (37,38), suppression of atrial natriuretic peptide (39), and direct renal effects of GH and IGF-I (40,41). Impaired thermoregulation (42-44) is also present in GHDA, partly attributable to reduced sweating capacity (11).

Insulin resistance – the epicenter of the metabolic syndrome - is not a central part of GHDA. GH antagonizes the effects of insulin on glucose metabolism in liver and skeletal muscle (45), and increased insulin sensitivity with fasting hypoglycemia is characteristic of childhood and adolescence GHD (46,47). The opposite is seen in active acromegaly (48). This effect of GH is rapidly reversible (49), and in normal physiology it operates in the fasting state when insulin activity is low (50,51). However, the prolonged biological half life of daily subcutaneous GH injections in the evening is unable to fully imitate the endogenous GH pattern (52), and GH replacement therapy invariably induces a certain degree of insulin resistance (51). Consequently, moderate elevations in the fasting levels of glucose and insulin are recorded in GHDA after GH replacement despite favorable changes in body composition (53).

The annual number of publications in the field of GHDA increased exponentially after 1989 from two to > 200 in 1999 (54), including dose-finding studies in different age groups (55-57) and it was confirmed that adult patients are highly sensitive to rhGH (58), and that male patients are more responsive to rhGH as compared to females (59,60). Collectively, this translated into guidelines for the clinical management of GHDA issued by the Growth Hormone Research Society (61). The indication for GH replacement in GHDA was approved by the European Union in 1994 alongside several other countries. Subsequently, meta-analyses of published data on adult GH replacement have been published on outcomes such as cardiovascular risk factors (53), muscle strength and exercise capacity (62,63), bone mineral density (64,65), body composition (66), and cardiac function (67). The meta-analyses confirmed and substantiated both the beneficial effects of GH

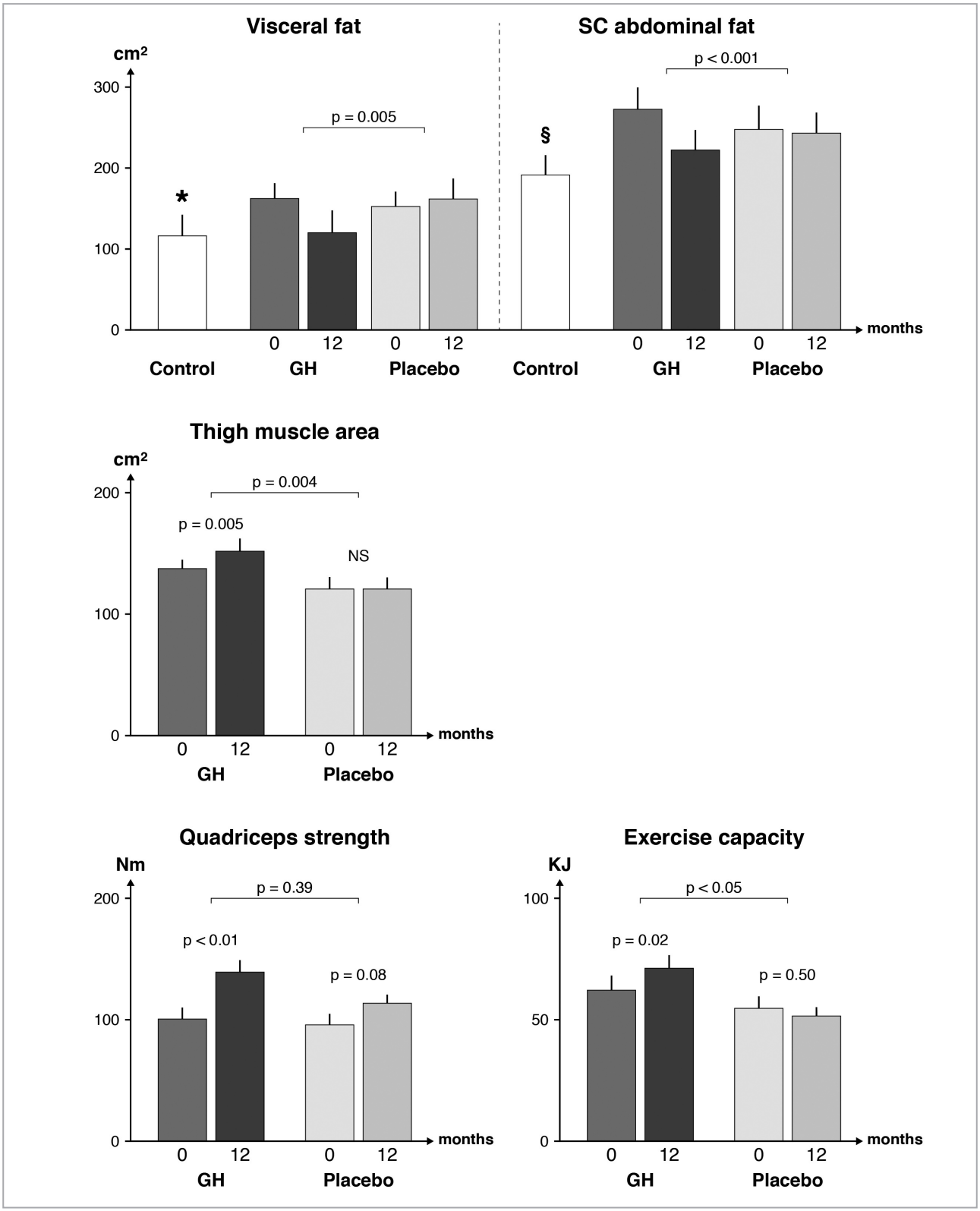

Figure 1. Effects of 12 months (t=0 and t=12 months) GH replacement vs. placebo in GHDA on visceral fat volume and subcutaneous abdominal fat (upper panel) as compared to a healthy reference group (control)
Mid panel shows change in thigh muscle area, and lower panel shows changes in isometric quadriceps strength and aerobic exercise capacity (bicycle ergometer). The data derive from Jorgensen et al (24).

replacement on body composition, bone mineral density, cardiac function and exercise capacity, as well as the side effects attributable to fluid retention and insulin resistance (53,66).

It remains uncertain if GH replacement therapy improves quality of life (QoL), since neither original studies nor meta-analyses provide unambiguous answers (66,68-70). Most QoL studies depend on questionnaires, which mainly record the patient's remembrance and do not detect day-to day experiences in real time. Of note, the most convincing beneficial effects of adult GH replacement on QoL was recorded in a placebo-controlled crossover study based on response from the spouse of the patient (71). Nevertheless, The National Institute for Health and Care Excellence (NICE) in the UK requires impaired pretreatment QoL in order to initiate adult GH replacement, and it also demands discontinuation of treatment in case of a lack of QoL improvement after 9 months treatment (72).

Transition and Senescence

Puberty marks the transition from childhood to adulthood, and represents a period with marked physical changes including a pubertal growth spurt and the development of secondary sexual characteristics leading to attainment of adult reproductive capacity. Muscle and bone mass increase markedly during and after puberty, which depend in part on amplified GH secretion and action resulting in grossly elevated IGF-I levels (73) (**figure 2**). The transition phase starts in late puberty when final adult height is attained (≈ mean age 15-17 years) and terminates in early adulthood when peak bone mass is reached (mean age ≈ 20-23 years).

In the era of pituitary hGH, GH replacement in childhood patients terminated when the target height was reached, but the introduction of rhGH made it feasible to continue treatment during the entire transition phase. An observational study reported that discontinuation of GH replacement in childhood-onset patients at the time of transition induced unfavorable changes in lipid profile and body composition (74), and a double blind, placebo-controlled parallel study has evaluated the effects of continuation vs. discontinuation of rhGH after cessation of linear growth (75,76). The latter study revealed that rhGH discontinuation resulted in decreased IGF-I as well as increased body fat and insulin sensitivity. After resumption of rhGH, lean body mass and IGF-I increased together with increased muscle volume of the thigh, muscle/fat ratio and reduced glucose oxidation rates (**figure 3**).

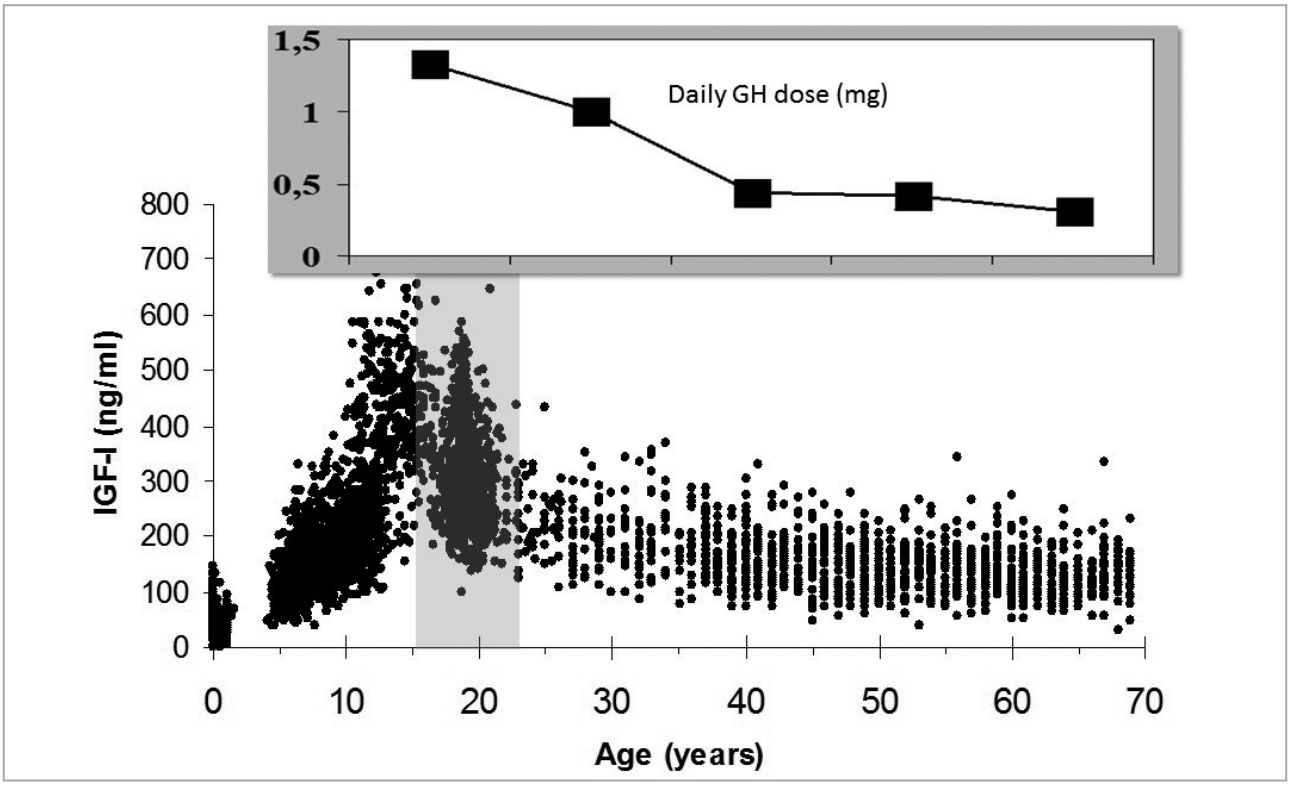

Figure 2. Serum IGF-I levels as a function of chronological age in 3851 healthy subjects of both sexes
The grey area indicates the duration of the transition phase. Modified from Juul et al. The insert illustrates the daily GH dose (mg) in GHDA to ensure a serum IGF-I level within the upper normal range for age as a function of chronological age. In addition to age, the GH dose also depends on gender.

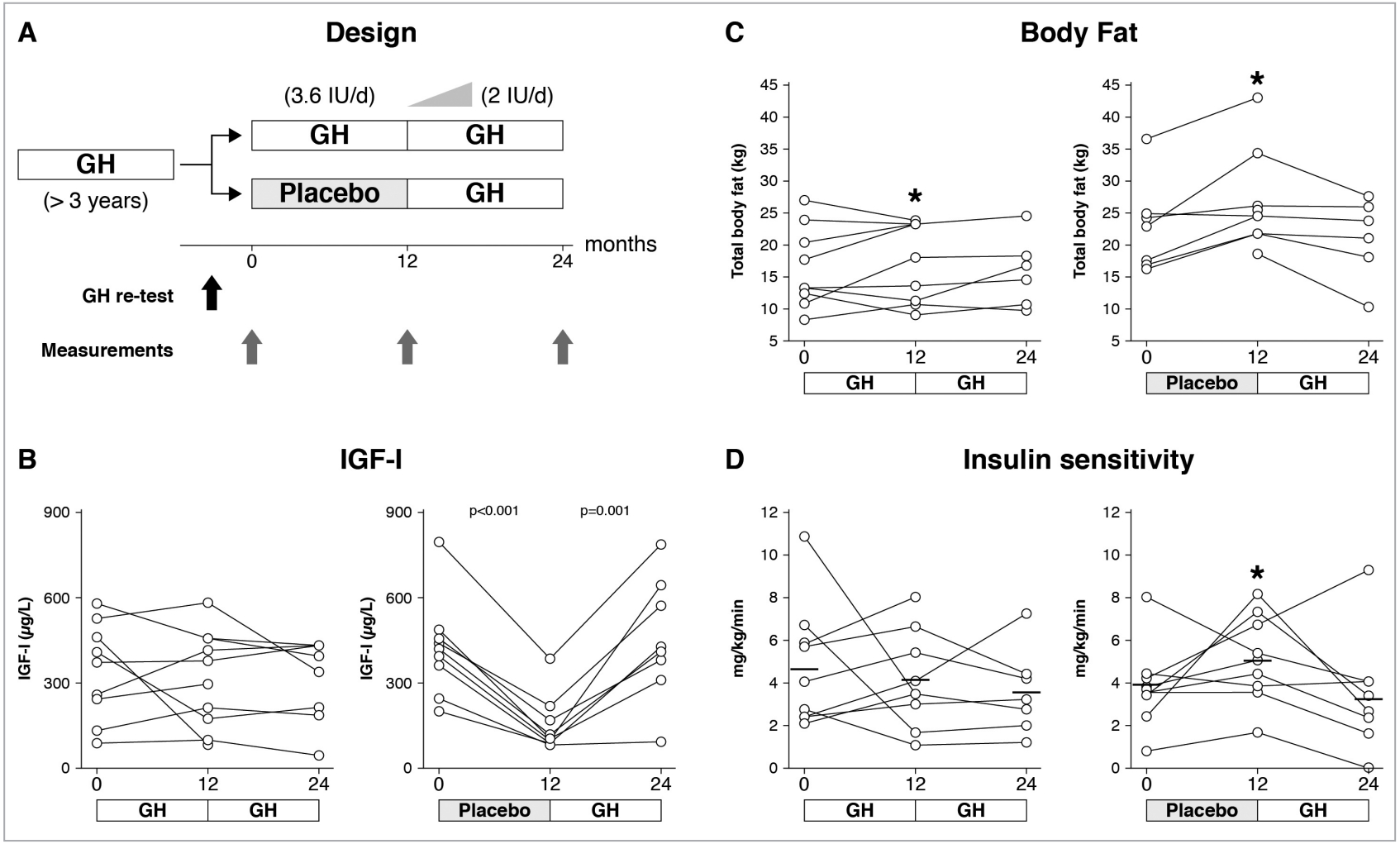

Figure 3. Placebo controlled continuation vs. discontinuation of GH replacement in the transition phase from childhood to adulthood in GHD
A. study design; the placebo-controlled phase was followed by 12 months open GH replacement.
B. individual changes in serum IGF-I levels
C. Individual changes in total body fat assessed by DXA
D. individual changes in insulin sensitivity assessed by the glucose clamp technique.
** indicates P < 0.05. The data derive from Vahl et al. (75) and Nørrelund et al.*

Comparable results were reported in an open study of continuation vs. discontinuation of GH replacement (77). A large proportion of GHD children exhibit normal GH secretion later in life, wherefore retesting is necessary unless there is strong evidence of either organic panhypopituitarism or a genetic cause of GHD (78). The pediatric mode of rhGH dosing according to body size translates into high daily doses to achieve the appropriate pubertal growth response and the normal high pubertal IGF-I levels. The proper management of transition patients includes patient involvement and a close collaboration between pediatric and adult endocrinologists (78).

Endogenous GH production and serum IGF-I levels decline with age (79) in parallel with senescent changes in body composition and physical performance (**figure 2**). Despite this decline in GH secretion, patients aged 60-80 years with documented panhypopituitarism still have reduced GH levels as compared to age-matched controls (80) and seem to respond to GH replacement in much the same manner as younger patients (81-83). However, the rhGH dose requirement in order not to induce supranormal IGF-I levels and to avoid side effects, declines with chronological age (**figure 2**).

Mortality

Increased mortality in hypopituitary patients due to cardiovascular disease is firmly established, but it remains uncertain if this is due unsubstituted GHD (25,84). Alternative or additional underlying causes include the underlying disease, treatment complications, and suboptimal substitution of additional pituitary deficiencies. It is also critical to understand that mortality and cancer incidence are increased in acromegaly (85,86), and that an inverse relation exists between activation of the IGF/insulin axis and longevity in many species (87). Moreover, epidemiological studies suggest an U shaped association between serum IGF-I levels and all-cause mortality in the general population (88).

Randomized studies of GH replacement therapy with mortality as an endpoint do not exist for good reasons, but observational studies in GHDA suggest that mortality is reduced in GH replaced patients as compared to GH-untreated patients (84,89-93) (**figure 4**).

Study	Year	Observed, *n*	Expected	SMR [95% CI]	Forest plot
Olsson *et al.*[90]	2017	93	80.2	1.16 [0.94-1.42]	
Svensson *et al.*[29]	2004	399	105.1	3.80 [3.43-4.19]	
Tomlinson *et al.*[26]	2001	181	96.7	1.87 [1.61-2.17]	
Bülow *et al.*[27]	1997	188	87.0	2.16 [1.86-2.49]	
Bates *et al.*[28]	1996	50	28.9	1.73 [1.28-2.28]	
Total				**2.14 [1.30-2.98]**	

2 3 4

Studies with GH treatment					
Study	**Year**	**Observed, *n***	**Expected**	**SMR [95% CI]**	**Forest plot**
Olsson *et al.*[90]	2017	29	44.4	0.65 [0.44-0.94]	
Gaillard *et al.*[93]	2012	528	465.5	1.13 [1.04-1.24]	
Van Bunderen *et al.*[92]	2011	95	74.6	1.27 [1.03-1.56]	
Svensson *et al.*[29]	2004	8	9.5	0.84 [0.36-1.66]	
Total				**1.00 [0.72-1.28]**	

2 3 4

Figure 4. Meta-analysis of published studies reporting mortality (SMR) in hypopituitary adults with and without GH replacement as an extension and update of data published by Pappachan et al. (84)
Forest plots showing the differences in SMR amoung hypopituitary adults without and with GH treatment
Studies without GH treatment

Discussion

rhGH therapy in GHDA is probably the best-documented therapeutic indication in pituitary endocrinology in terms of placebo-controlled trials and observational studies. The favorable change in body composition with reduced fat mass and increased lean body mass is the most robust effect. Most studies also record improvements in aerobic exercise capacity and cardiac function. As regards bone mass and strength, data from placebo-controlled trials for up to one year record increased bone turnover but unchanged or even reduced bone mass (94), whereas a study of 18 months observed a significant increase in BMD (95). It is assumed that GH replacement initially increases bone remodeling, which transiently reduces BMD, followed by a moderate but sustained increase at least in male patients (64). It is uncertain if this reduces the risk of osteoporotic fractures, but observational studies suggest this to be the case (96). Whether quality of life improves remains uncertain, since the results from placebo-controlled trials are ambiguous, and the results from open and observational studies are likely biased. The level of education is similar among GH treated adult patients as compared to the background population, but a higher proportion of the patients are unemployed, retire earlier and are less likely to live with a partner (89,97). These socioeconomic outcomes demonstrate that hypopituitarism remains a clinical challenge. Cancer risk is not increased with rhGH treatment, and mortality (98), if anything, is reduced (89,90) (**figure 4**). The latter observation is reassuring even though selection bias is a likely contributor.

Side effects in terms of fluid retention and impaired insulin sensitivity do occur, but they are benign, dose-dependent, and rapidly reversible. Still, it is important to avoid overtreatment. The daily GH dose requirement to avoid supernormal IGF-I levels and side effects is 0.1 mg or lower in male patients aged ≥ 70 years, which is only 10% of the dose used in the early adult trials. In this regard, it should be mentioned that the performance of serum IGF-I as a biomarker of rhGH treatment is not very good (99), and the need for a better biomarker increases with the introduction of long acting rhGH preparations, which result in widely fluctuating serum IGF-I levels (100).

The first anecdotal report of GH treatment of a hypopituitary adult patient in 1962 concluded that 'observations will be needed in more cases to indicate whether the favorable effect was more than coincidental' (3). Today, it is safe to reply that observations from numerous placebo-controlled trials confirm beneficial effects and justify the treatment.

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Growth Hormone Therapy for Turner Syndrome

Christopher Blunden, MD, Nat Nasomyont, MD, Philippe Backeljauw, MD

Cincinnati Children's Hospital Medical Center Division of Pediatric Endocrinology

Corresponding author: Christopher Blunden, MD, 3333 Burnet Ave, MLC 7012 Cincinnati, OH 45229, USA, Tel: 513-803-1429, Fax: 513-803-1174, E-mail: christopher.blunden@cchmc.org

Abstract

Growth failure is nearly universal in individuals with Turner syndrome (TS). It is a consequence of haploinsufficiency of the short stature homeobox gene located on the short arm of the X chromosome (SHOX). Without treatment, individuals with TS are expected to be on average 20 cm shorter than unaffected adult females. Short stature is cited by patients as one of their biggest burdens and may have an adverse impact on psychosocial well-being, pubertal timing, and ability to complete a variety of daily living activities. The routine use of recombinant human growth hormone (rhGH) treatment has increased height outcomes. Clinical evidence has strongly supported the efficacy and safety of this treatment. In this article we review the rationale for rhGH treatment in TS, the factors that affect treatment response, safety and monitoring considerations, and potential changes in the way rhGH may be utilized in TS care in the future.

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***Key words**: Growth hormone, Turner syndrome, SHOX haploinsufficiency*

Introduction

Short stature is often one of the most striking phenotypic features of Turner syndrome (TS) and is therefore a common reason for endocrinology referral prior to or after diagnosis. The reason for suboptimal linear growth lies primarily in the decreased genetic dosage of the short stature homeobox (SHOX) gene located in the pseudoautosomal (PAR1) region of the short arm of the X chromosome (Xp22.3) (1). SHOX is part of a large evolutionary family of transcription factors that play a role in developmental regulation. More specifically, SHOX acts as a transcriptional activator in osteogenic cell lines (2). There is ample evidence that SHOX is critical for growth. For example, it is located in the critical region on the X chromosome that escapes X-inactivation, and mutations or deletions are likely to exert a dosage effect (3). In addition to being a homeodomain protein, the SHOX gene is expressed at the highest level in bone morphogenic tissue (4). When SHOX haploinsufficiency occurs there is decreased chondrocyte proliferation and differentiation at the growth plate, leading not only to short stature, but also cubitus valgus, Madelung deformities and other common skeletal features of TS (4).

There are several periods during which linear growth is negatively affected in TS. *In utero*, individuals with TS may display a mild degree of growth retardation. One study of 24 mid-trimester 45,X and 45,X/46,XX fetal specimens demonstrated growth deficiency of all long bones (5). A large study from the French National Rare Disease Network described the growth parameters of 1,037 TS infants with a mean gestational age of 39 weeks (6). The results are summarized in **table 1**. TS infants with karyotype X,r(X) were most affected by intrauterine growth restriction while infants with mosaic 45,X/46,XX karyotype were least growth restricted. In all TS infants, the mean birth length standard deviation score (SDS) was -1.16, the mean birth weight was -0.90 SDS and the mean head circumference was -0.72 SDS. The prevalence of small for gestational age (SGA) was 24% for birth length and 19% for birth weight for all infants with TS.

Table 1. Growth parameters of TS infants at 39 weeks gestational age in SDS (Modified from Fiot, 2016)

	All TS n = 1037	45,X n = 371	X,r(X) n = 77	45,X/46,XX n = 153
Birth Length	-1.16	-1.16	-1.55	-0.71
Birth Weight	-0.90	-0.88	-1.53	-0.64
Birth Head Circumference	-0.72			

Postnatally, there continues to be slow growth, which can be further exacerbated by decreased caloric intake if there are feeding difficulties due to a high arch of the palate. Many reports have documented a slowed childhood component of growth in individuals with TS. One study followed 37 untreated infants with TS and showed a decline in mean height SDS from -0.5 at birth to -1.5 at age 12 months and -1.8 at age 18 months (7). A randomized controlled study of recombinant human growth hormone (rhGH) therapy in early childhood demonstrated a decline in height SDS of 37 untreated children with TS between ages 2 to 4 years from -1.76 to -2.16 SDS (8). In addition, 1,075 individuals in the French cohort described above were followed until mean age 8.8 years without rhGH treatment and they showed a further decline in height SDS to -2.60 when compared to the general female population (6). These findings are consistent with the Pfizer International Growth Study (KIGS) registry wherein 987 participants had a mean height SDS of -2.4 at age 9.7 years - before rhGH treatment was initiated (9).

Finally, untreated individuals with TS fail to demonstrate a normal pubertal growth spurt, with prolonged slow linear growth into late adolescence or early adulthood. Adult women with TS never treated with growth promoting therapies can be expected to be 20 cm shorter (approximately -3 SDS) than the average unaffected female with two intact functional copies of the SHOX gene (10).

History and Rationale of rhGH Treatment in TS

Although patients with TS are not growth hormone (GH) deficient, based on data from the last three decades rhGH has been found to be a safe and efficacious supplemental treatment to partially overcome the effect of SHOX haploinsufficiency on growth. The goal of rhGH therapy is to facilitate attainment of a height during childhood and adulthood that minimizes physical restrictions and allows puberty to be initiated at the appropriate time for optimal bone mineralization and for psychosocial and cognitive benefits. Evidence of increased height outcomes in combination with a low risk profile has led to rhGH therapy being approved by regulatory authorities (United States Food and Drug Administration, USFDA – 1996, European Medicines Agency, EMA – 2001) and being fully recommended in international clinical guidelines as standard of care in early childhood TS treatment (11). SHOX haploinsufficiency without TS is a separately approved indication for rhGH treatment with similar height outcomes.

Studies have reported variable degrees of quality of life (QoL) improvement after rhGH therapy, in part due to various methodologies. One study of 49 individuals with TS at mean age 19.6 years who were former participants of the Dutch GH Dose Response Trial (12), and had puberty induction at an age-appropriate time, reported a normal health-related QoL on the Short Form 36 Health Survey (SF-36) and TNO/AZL Adult Quality of Life (TAAQOL) evaluations when compared to non-TS peers, including in the domains of social functioning and physical functioning/daily activities (13). However, another study of 111 adult TS women of age 18-59 years compared to randomly selected age-matched non-TS women showed more social isolation in TS women than controls despite a mean 5.1 cm height gain from rhGH treatment (14). There was no other significant impact on QoL attributable to rhGH treatment using the Psychological General Well-Being scale and Nottingham Health Profile (14).

Growth hormone therapy has been shown to lead to modest increases in adult stature for most individuals with TS. Despite the existence of many reported studies of rhGH treatment in TS, a 2007 Cochrane Center review (15) identified only four trials in which rhGH treatment was compared in a randomized fashion with a concurrent non-treatment or placebo-controlled group for at least one year (16-19), and only a single randomized controlled trial that followed participants to adult height (16). More recently, a 2-year randomized controlled trial evaluating the impact of GH initiation at a younger age (before 4 years), and one double-blind, placebo-controlled trial to adult height have been published (8,20).

Efficacy of rhGH Treatment in TS

Early trials of rhGH therapy in young girls with TS took place in the 1980s (17,21-23). A randomized controlled trial of

methionyl GH (metGH) administered to individuals with TS ages 4-12 years was analyzed at 1, 3, and 6-year follow-up intervals. Seventy patients were randomly assigned to receive either no therapy, or metGH 0.125 mg/kg three times per week, oxandrolone 0.125 mg//kg/day, or combination metGH and oxandrolone therapy. One-year data revealed improved growth velocity (GV) from 3.8 cm/yr in controls to 6.6 cm/yr in the metGH only group, and 9.8 cm/yr in the metGH plus oxandrolone group. This correlated with an increase in predicted adult height (PAH) of 2.5 cm in the metGH only group and 3.2 cm in the combination group. After the initial study period, the control and oxandrolone only groups were transitioned to combination therapy. At a three-year interval, the metGH only group had a GV of 4.6 cm/yr and had an improved PAH of 4.5 cm. The combination group had a GV of 6.1 cm/yr and an increased PAH by 8.2 cm. Six-year data from 62 subjects on metGH and combination therapy revealed 30 subjects that had completed treatment with a mean height 8.1 cm above their PAH and, including the 32 subjects still being treated, 55/62 that had exceeded their PAH (14/17 in the metGH only group). The GV of treated individuals remained above that anticipated for untreated individuals throughout the trial. In 1998 a follow-up study described the outcomes of 17 rhGH only treated patients and 45 patients on combination therapy who had completed treatment and compared them to a 25 patient American historical control group (24). The mean height gain as compared to baseline PAH was 8.4 cm in the rhGH only group, 10.3 cm in the combination group, and 0.0 cm in the control group.

In 2000 a cohort of 686 patients with TS from KIGS who received rhGH treatment for 1 to 4 years was analyzed by multiple regression analysis for predictors of treatment response (25). The study identified rhGH dose, younger age at onset of treatment, and response to treatment during the first year as positive predictors of growth response. As part of the validation process for a mathematical height prediction model, 76 additional rhGH-treated patients with TS from KIGS were compared to a German cohort of 81 rhGH-treated TS patients. Initiated on a mean rhGH dose of 0.95 IU/kg/week and 0.70 IU/kg/week at age 8.2 and 9.5 years, respectively for the KIGS and German cohorts, first year GV was 7.8 cm/yr and 7.5 cm/yr, second year GV was 6.5 and 6.3 cm/yr, third year GV was 5.9 and 5.7 cm/yr, and fourth year GV was 5.3 and 5.4 cm/yr.

The above trials ultimately provided the basis on which rhGH became approved for routine clinical use in TS by the USFDA in 1996 and by the EMA in 2001.

In 2002, an American randomized placebo-controlled trial replicated the results of early rhGH trials (18). 232 patients with TS received rhGH at 0.27 mg/kg/week, 0.36 mg/kg/week or placebo in addition to low dose oral estrogen or placebo. During the 18-month placebo-controlled portion of the trial, patients treated with only the lower dose of rhGH had a GV of 6.6 cm/yr compared to 6.8 cm/yr in those treated with the higher dose and 4.2 cm/yr for the placebo group. In treated patients, near-adult height (NAH) after an average of 5.5 years of rhGH treatment starting at mean age 10.9 years was an average of 1.3 SD above the TS standard. Of those treated, 29% exceeded 152.3 cm (60 inches, 5 feet 0 inches), whereas only 5% of untreated TS women are expected to attain an adult height of 60 inches. This trial also showed correlations between height outcome and earlier age at rhGH initiation, as well as taller stature at treatment initiation.

In 2003 a Dutch trial expanded on previous studies by analyzing the dose-response of 60 girls with TS randomized into groups receiving 45 mcg/kg/day of rhGH throughout the trial, 45 mcg/kg/day for one year then 67 mcg/kg/day, and 45 mcg/kg/day for one year, then 67 mcg/kg/day for another year, and 90 mcg/kg/day from the third year onward (12). Individuals remained on treatment for a minimum of 4 years prior to sex steroid replacement therapy initiation at mean age 12.7 years, and the mean duration of rhGH therapy was 8.6 years. The 'standard' treatment group (45 mcg/kg/day at all times) achieved an 11.9 cm mean height gain compared to their PAH before treatment. A 15.7 cm mean height gain was seen in the 67 mcg/kg/day group. Even more height gain (16.9 cm) was observed in the highest dosing group, but at the expense of IGF-I concentrations above +2SDS. In all treatment groups, 50/60 individuals achieved a normal adult height, defined as a height SDS above -2 on standard female population growth curves. This trial underscored the dose – and treatment duration dependent nature of rhGH treatment (**figure 1**), and laid the basis for the recommended dose escalation to 67 mcg/kg/day in selected patients.

The first randomized trial in TS of rhGH therapy fully controlled to adult height was conducted in Canada and published in 2005 (16). 104 pre-pubertal girls with TS ages 7-13 years were treated with 50 mcg/kg of rhGH six times per week with standardization of sex steroid replacement initiation at age 13 years. Patients completing the protocol remained on treatment for a mean of 5.7 years. At bone age 14 years GH-treated patients were a mean 6.5 cm taller than controls. At 1-year follow-up treated individuals were a mean 6.8 cm taller than controls (**figure 2**).

The first randomized controlled study involving the treatment of toddlers with TS was published in 2007 (8). Starting at age 2 years, 45 patients received 50 mcg/kg/day of rhGH and 43 patients received no treatment. Of the individuals who completed 2 years of measurements, a 1.6 SDS between-group difference was observed, providing strong evidence for early initiation of rhGH treatment (**figure 3**). 69 individuals, 36 of whom were in the early treatment group, participated in an extension of the study starting at age 8.5 years receiving rhGH at an average of 41 mcg/kg/day (26). Data from this extension study, presented at the 2016 Endocrine Society

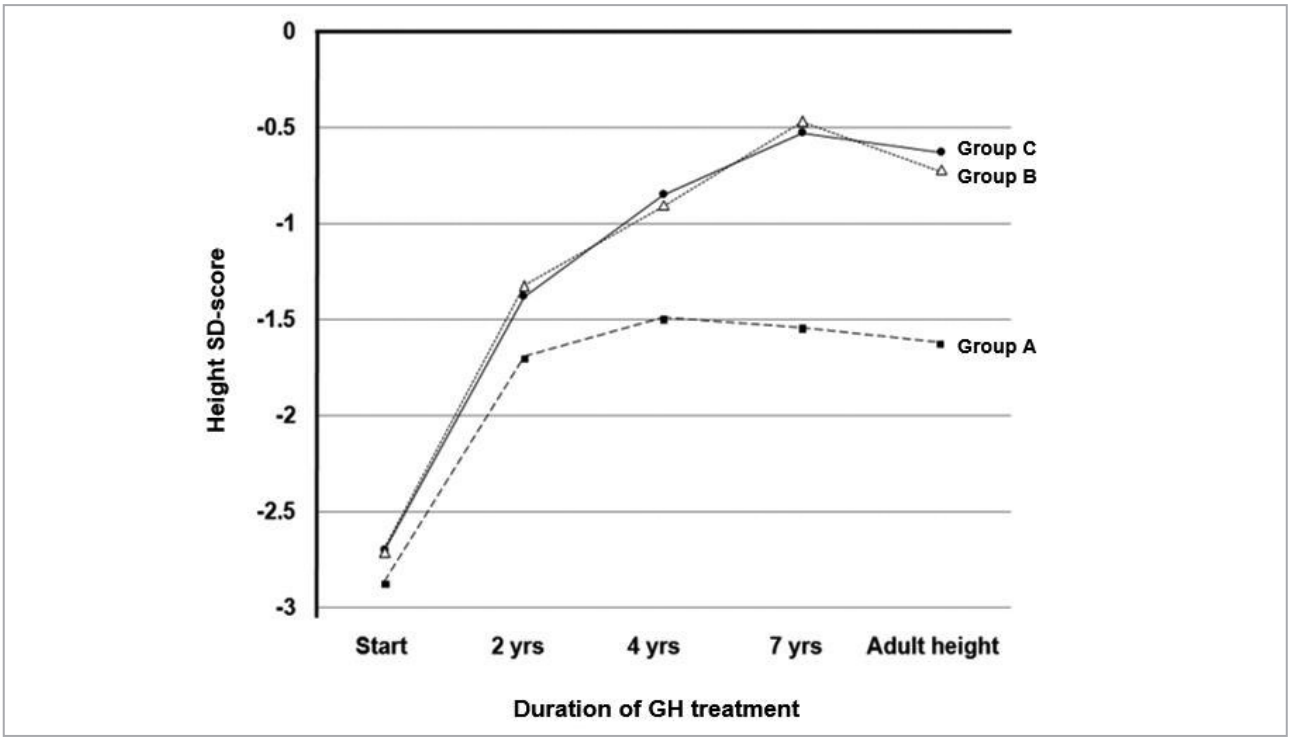

Figure 1. Mean height SDS over time on rhGH therapy for Dutch girls with TS treated with rhGH at 45 mcg/kg/day for 7 years (Group A), 45 mcg/kg/day for 1 year then 67.5 mcg/kg/day for 6 years (Group B), and 45 mcg/kg/day for 1 year, then 67 mcg/kg/day for 1 year, then 90 mcg/kg/day for 5 years (Group C) (Modified from van Pareren, 2003)

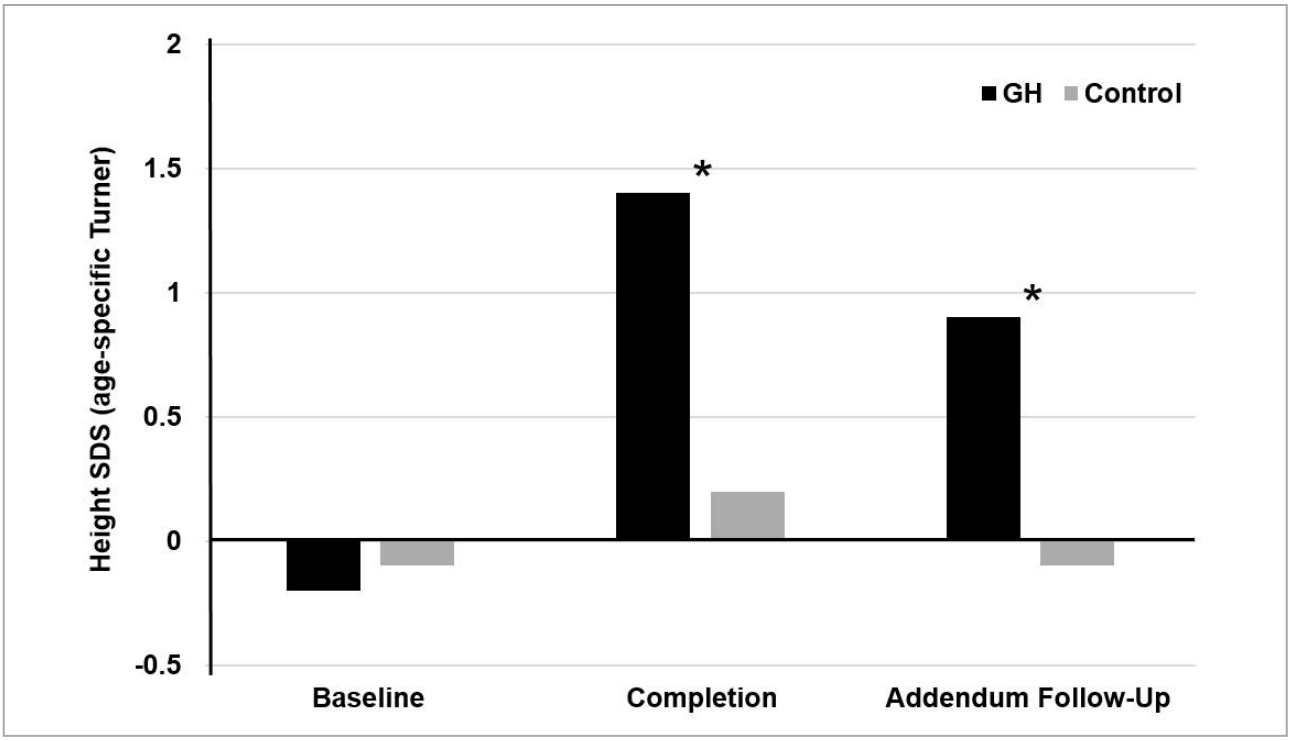

Figure 2. Mean height SDS (age-specific Turner) of young patients with TS treated with rhGH 50 mcg/kg/day and untreated controls at baseline, near-adult height, and at >12 months after reaching near-adult height plotted on TS growth curves (Modified from Stephure, 2005)

**p <0.001 based on least-squares means differences in ANCOVA model*

Annual Meeting, showed that the early treated group had a baseline height SDS -0.68 versus -1.29 in the early untreated group. The near adult height SDS was -1.37 at age 14.6 for the early treated group and was -1.60 at age 15.3 years for the 'early untreated' group.

By 2007, the KIGS database had followed 1,146 individuals with TS treated with rhGH to adult height (27). At mean age 16.8 years with mean total treatment of 7.1 years and mean GH dose at rhGH start of 0.28 mg/kg/week, an adult height SDS of -2.3 was achieved compared to a baseline height SDS of -3.0.

In 2010, Dutch patients with TS were treated with rhGH in a randomized placebo-controlled double-blinded trial to evaluate the dose response of rhGH when combined with different doses of oxandrolone therapy (28). In the rhGH only group of 42 patients treated with 46 mcg/kg/day starting treatment at mean 9.4 years (with estrogen replacement starting after age 12 years) and mean treatment discontinuation age of 15.8 years, an adult height gain of 7.2 cm was observed compared to baseline PAH, correlating with a baseline height SDS of -3.0 that improved to -2.3 SDS at the end of the trial.

In 2011, a double-blind placebo-controlled trial was conducted to assess the outcomes of rhGH and low-dose estrogen replacement in childhood in TS (20). In similar fashion to the 2002 trial outlined above, 149 patients were randomized to rhGH treatment (100 mcg/kg three times per week), low dose estrogen treatment, combination therapy, or double placebo. Pubertal sex steroid replacement therapy was initiated after age 12 years. At 17.0 years after a mean treatment duration of 7.2 years, rhGH therapy only patients attained a mean height of -2.29 SDS compared to a -2.81 SDS in controls and -2.10 in the combination therapy group. The overall treatment effect of rhGH on adult height was 5.0 cm (+0.78 SDS).

There is consistently wide variation in individual treatment response and outcomes after rhGH therapy in girls with TS. In general, evidence is strong that there is a 5-7 cm adult height gain from rhGH treatment when compared to untreated controls and a 7-12 cm adult height gain from baseline PAH (11). An individual patient's height outcome after rhGH treatment is determined by multiple factors, which are accounted for in Ranke's height prediction model, developed from an analysis of 987 patients from KIGS who had been on rhGH for at least 4 years (9). Through multiple regression analysis, a model was developed for near adult height and total height gain. The TS karyotype was found to have no influence on outcome. In order of importance, improved outcomes were seen with: **1.** being taller at the start of rhGH treatment, **2.** better responsiveness during the first year of treatment, **3.** taller genetic potential, **4.** older

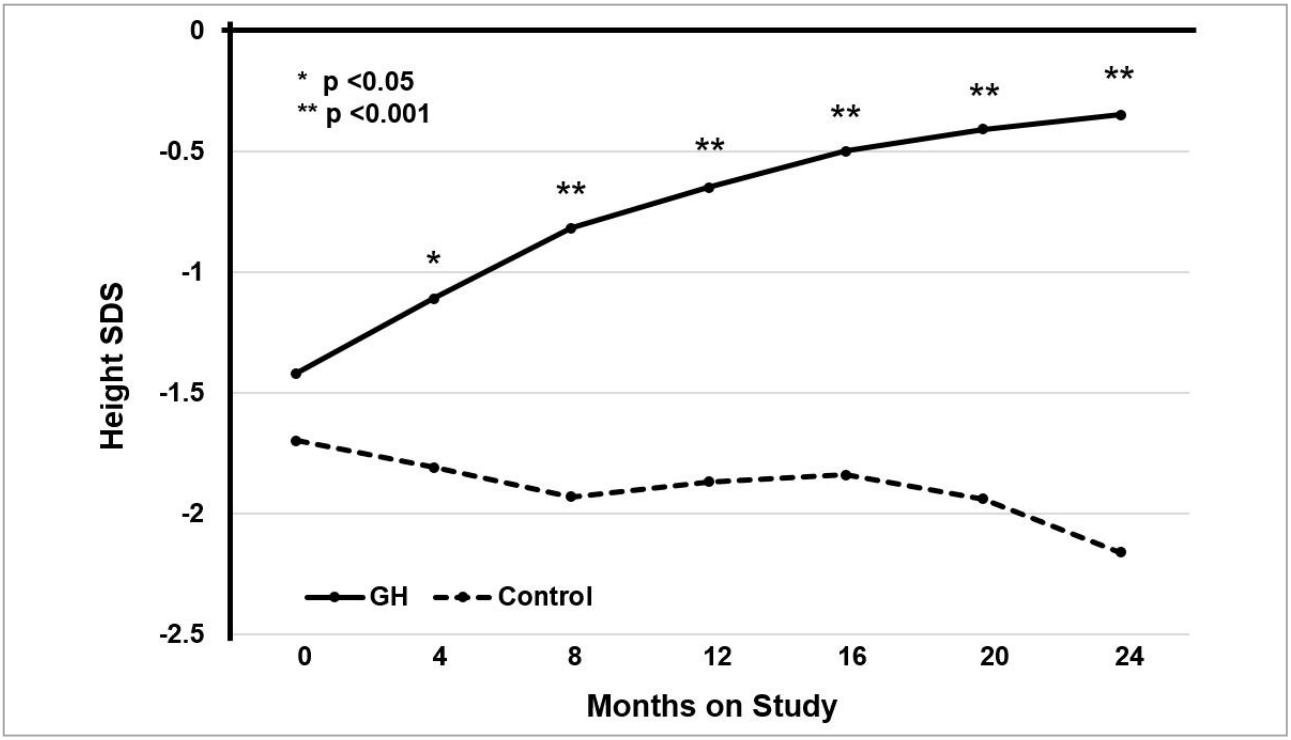

Figure 3. Mean height SDS of patients with TS treated with 50 mcg/kg/day of rhGH and untreated controls over a 24 month period from ages 2 until 4 years *(Modified from Davenport, 2007)*

age at pubertal onset, **5.** younger age at rhGH treatment initiation, and **6.** higher mean dose per week. The regression equation NAH (cm) = 142.9 + [MPH (SDS) x 1.37] + [height at GH start (SDS) (TS) x 4.11] + (studentized residual 1st year x 1.99) + [mean GH dose (mg/kg/wk) x 4.82] + (age at puberty start yrs x 0.74) explained 67% of the variability of (near) adult height with an error SD of 3.6 cm.

Recommendations for rhGH Treatment in TS

Current international clinical practice guidelines suggest initiating rhGH therapy at 4-6 years of age and continuing therapy until a bone age of 14 years, height velocity <2 cm/year or until the patient is satisfied with her height (11). Although all countries recommend treatment seven days per week, different regions of the world initiate treatment at slightly different doses (**table 2**).

Table 2. Typical starting doses of rhGH for the treatment of short stature in Turner syndrome in several regions of the world

Location	Typical rhGH Starting Dose
North America	50-54 mcg/kg/day
Europe	45-50 mcg/kg/day
Australia	21-45 mcg/kg/day
*In those patients with a late diagnosis, who are pubertal, or who have extreme short stature a dose up to 68 mcg/kg/day may be considered.	

Safety Considerations of rhGH Therapy in TS

Growth hormone therapy is generally well tolerated in individuals with TS and most reported adverse effects appear to be uncommon.

Intracranial Hypertension (IH)

When occurring, IH generally develops within several weeks to one year after rhGH initiation, but may be seen at any time during the course of treatment (29). Symptoms of IH may include persistent headaches, papilledema, and visual field changes. In a retrospective study of 6,092 patients with TS in the KIGS database, the incidence of IH was 56.4 per 100,000 treatment years, with median time to adverse event of 0.8 years (29). The frequency was 197 per 100,000 patients, which was higher than 6 of 7 other described patient groups, including those with GH deficiency and idiopathic short stature (ISS). These results were consistent with another large retrospective analysis of 5,127 patients with TS from the National Cooperative Growth Study Database (NCGS) which described an IH incidence of 0.2% (30). IH typically subsides with discontinuation of rhGH administration, but procedural interventions such as a lumbar puncture to siphon off cerebrospinal fluid and relieve pressure may be required in severe cases. After resolution of symptoms, rhGH treatment may be restarted at a lower dose with close observation.

Musculoskeletal System

Data from the same KIGS and NCGS observational studies showed that patients with TS have an incidence of slipped capital femoral epiphysis (SCFE) of 84.5 per 100,000 treatment-years with an incidence of 295.5 per 100,000 patients (KIGS) and 0.2% (NCGS) (29,30). This was again higher than the incidence in patient groups with GH deficiency and ISS. Additional risk factors for development of SCFE include heavier body weight and older age (31).

Scoliosis also has been reported to be more common in rhGH-treated TS patients compared with other rhGH-treated children, with an incidence of 0.7% (30). This may be because children with TS are known to have higher incidence of idiopathic scoliosis even without exogenous GH exposure (32,33). It is also possible that rapid increases in linear growth from rhGH administration may accelerate the progression of existing scoliosis in these patients (34).

Body Proportions

Girls with TS have disproportionate short stature, which is characterized by short extremities in relation to trunk and relatively large hands and feet (35,36). Body proportions were assessed in the 7-year Dutch Dose-Response Trial in 68 participants and demonstrated moderate improvement in height-to-sitting height ratio after rhGH treatment, but increasingly disproportionate large feet over time, more pronounced with higher doses (37). Development of large feet may influence the decision to discontinue rhGH treatment earlier in some patients with TS.

Insulin Resistance and Carbohydrate Metabolism

Data from a cross-sectional study of 13 women with TS and 13 BMI-matched controls administered oral and IV glucose tolerance tests as well as euglycemic and hyperglycemic clamps has identified normal insulin sensitivity, but a significantly delayed ß-cell response and glucose intolerance in young women with TS (38). Concerns for increasing insulin concentrations with rhGH treatment have been raised because of the predisposition to type 2 diabetes mellitus. Data from a prospective rhGH frequency administration-response study demonstrated increased insulin concentrations during oral

glucose challenge testing before, during and 6 months after a mean 43 months of rhGH treatment, indicating insulin resistance, but no significant change in glycemia (39). In a short-term follow-up to the Dutch Dose-Response Trial, fasting glucose returned to pretreatment values after 6 months and fasting insulin values decreased to just above pretreatment concentrations without any dose-dependent differences (40). Only 1 of 53 patients had impaired glucose tolerance after 6 months off rhGH treatment. Long-term follow-up at a mean 4.8 years post-treatment demonstrated persistent elevation of fasting insulin concentrations (41). A recent longitudinal study also found no negative effect of rhGH treatment on glucose tolerance despite relative insulin resistance (42).

Despite overall reassuring long-term outcomes following rhGH treatment, current recommendations are to monitor carbohydrate metabolism during and after rhGH treatment as it is still unclear whether rhGH treatment increases the risk of type 2 diabetes mellitus (43).

Body Composition and Lipid Metabolism

Long-term data was collected from 39 participants in the Dutch Dose-Response Trial to age 20 years, with lipid concentrations before rhGH treatment, after 4 years of rhGH therapy, 6 months after discontinuing rhGH therapy, and at 4.8 years after rhGH discontinuation (41). At baseline, the atherogenic index in patients with TS was higher than 703 normal Dutch female controls (6.2 vs. 3.6), primarily due to lower HDL concentrations. However, HDL significantly increased on rhGH therapy and continued to rise even after treatment was discontinued. This resulted in a 50% improvement in atherogenic index from baseline to long-term follow-up (6.2 to 3.1). Total cholesterol and LDL also significantly decreased on rhGH treatment, but this effect did not persist. Higher LDL and total cholesterol were associated with longer time off rhGH treatment. Triglyceride concentrations significantly increased on rhGH therapy and remained stable at long-term follow-up.

A cross-sectional study of 76 rhGH-treated individuals with TS (mean treatment duration 4.0 years) compared to 26 untreated controls with TS showed significantly lower visceral and subcutaneous abdominal adiposity as well as total body fat in the rhGH-treated group (44). This result led the authors to suggest that the beneficial effects on body composition observed with rhGH treatment may outweigh the transient increase in insulin resistance that occurs.

Cardiovascular System

There is an increased risk of hypertension and arteriosclerosis in individuals with TS, and cardiovascular disease accounts for up to 50% of deaths in adults occurring 6-13 years earlier than expected (45). The prevalence of arterial hypertension has been reported to be 25% in pediatric patients with TS (46). Long-term blood pressure data from the Dutch Dose-Response Trial was collected at baseline, and after 2-years, 4-years, and 7-years of rhGH treatment, as well as 6-months post-treatment (40). Results demonstrated an unchanged systolic blood pressure SDS in all groups during treatment with a significant decrease after treatment discontinuation. In all groups, a significantly decreased diastolic blood pressure was observed during treatment, with stabilization after treatment discontinuation. While blood pressure SDS for both systolic and diastolic readings remained higher than the normal population throughout the trial, rhGH therapy did seem to confer overall BP benefit in patients with TS (40).

A retrospective cross-sectional study in rhGH-treated and untreated individuals with TS showed no influence of rhGH on ascending or descending aortic diameter (47). In addition, cardiac magnetic resonance imaging comparing 38 former participants in the Dutch Dose-Response Trial at mean age 12 years with 27 healthy controls at mean age 21 years showed rhGH-treated patients with TS had dilated aortas and impaired wall distensability compared to controls, but those treated with higher rhGH doses had less severe abnormalities (48). A total of 5 deaths from aortic dissection in 5,127 individuals with TS were reported in over 20 years of NCGS data collection while monitoring the safety of rhGH treatment (30).

Neoplasia Risk

It has been suggested that women with TS are at increased risk for some forms of cancer, including meningioma and childhood brain tumors (49,50). There is also an epidemiologic association in non-TS individuals between supraphysiologic IGF-I concentrations and some forms of neoplasia (51). However, currently there is no evidence to suggest that a supraphysiologic IGF-I value induced by rhGH therapy is associated with increased risk for childhood or adult cancer in TS, or with increased mortality in any rhGH treated patient population (52). In 54,996 children on rhGH therapy for various indications in the NCGS database over 20 years, no increased risk of de novo malignancy was reported (30). Current recommendations for IGF-I concentrations in rhGH-treated TS patients (detailed below) are therefore based on risk-benefit assessments and an abundance of caution as data on long-term cancer incidence in rhGH exposed populations is not yet available. Active malignancy is an absolute contraindication to rhGH treatment in any patient population.

Monitoring rhGH Treatment in TS

Recommendations for the monitoring of rhGH treatment in individuals with TS are included in recently published international clinical practice guidelines (11). Individuals with TS on rhGH therapy should have a height measurement every 4-6 months and an IGF-I level monitored at least annually,

though additional measurements should be considered before initiating treatment, at first follow-up, and with significant dose changes. The purpose of IGF-I measurement is for monitoring compliance and guidance in titrating rhGH dosing to achieve optimal linear growth while avoiding persistently high IGF-I concentrations associated with short and long-term adverse effects (53). A retrospective study of individuals with TS treated with rhGH for 1 year in the KIGS database revealed a positive correlation between rhGH dose and IGF-I SDS (r = 0.25), a positive correlation between pre and post-treatment change in IGF-I and increase in height SDS (r = 0.24), and significantly increased GV among patients with an IGF-I SDS between +1 and +2 as compared to those with on-treatment IGF-I concentrations between -1 to -2 SDS (53). In the Dutch Dose-Response Trial over 7 years, those in the standard dosing group (45 mcg/kg/day) regularly had a mean IGF-I SDS of +1 to +2, while the higher dosing groups were regularly maintained in the +2 to +3 range without any treatment-related adverse events reported (12). In the Toddlers with TS study over a 2-year period, 37% of 45 rhGH-treated subjects had at least one IGF-I value above +2 SDS, but no new or unexpected safety signals associated with treatment were detected (8). Therefore, treatment target IGF-I levels should be within +2 SDS of the mean for age (the normal range) with a value above +3 SDS being an indication for dose decrease (11). An intermediate IGF-I between +2 and +3 SDS for age warrants clinical judgment to be applied with consideration to height SDS and GV as there is a lack of evidence and agreement about risks and benefits associated within that range. Clinical evaluation for scoliosis should be completed every 6 months and routine screening for symptoms of IH and SCFE should always be done.

Adjunct Growth-Promoting Treatments in TS

Oxandrolone

The anabolic non-aromatized steroid oxandrolone, in combination with rhGH therapy, has been shown to increase adult height in patients with TS. An approximately 2-5 cm adult height gain may be observed when compared with rhGH treatment alone (24, 54-57). Oxandrolone may be considered in patients with TS who initiate rhGH treatment at older ages or if the PAH is lower than desired/expected. Recent clinical practice guidelines suggest initiating oxandrolone therapy around age 10 years with a starting dose of 0.03 mg/kg/day, and to only titrate the dose to a maximum of 0.05 mg/kg/day (11). Delayed breast development and virilization (e.g. clitoromegaly, hirsutism, acne and voice deepening) are adverse effects from oxandrolone that can be avoided by using only this dosing range. It should be noted that the availability of oxandrolone depends from country to country.

Ultra-Low Dose Estrogen

The previously described double-blind, placebo-controlled trial of rhGH with very low-dose estrogen in prepubertal patients with TS demonstrated a modest synergetic effect of concomitant estrogen and rhGH on adult height (20). Adult height was greater in the combination therapy group than rhGH only group by 0.32 SDS (2.1 cm). The difference did not reach statistical significance (p = 0.059). Such ultra-low-dose estrogen treatment is currently not recommended for routine use in prepubertal TS patients because of limited data on dosing, route of administration and long-term safety.

Future of rhGH Treatment in TS

Poor adherence to rhGH treatment commonly contributes to suboptimal linear growth in girls with TS. A national survey of rhGH compliance in New Zealand showed that of 175 patients, 27% of whom had TS, 66% missed more than one injection per week over a four-month period according to rhGH vials returned (58). Another study of only 6 individuals with TS found a mean 86.0% adherence rate when recorded by an electronic auto injector device over a 1.5 year period (59). In an observational study from 1990-2013 in Australia, 34.8% of 626 individuals with TS prescribed rhGH treatment were classified as having “early cessation of treatment”, indicating they had not met criteria by bone age or height as having “completed treatment” and were not “non-responders” (60).

Long term treatment and discomfort associated with daily injections are two factors contributing to non-adherence and early cessation of daily subcutaneous rhGH therapy (58). Long-acting rhGH preparations may offer benefit compared to conventional daily rhGH as they reduce the inconvenience of daily injections (either weekly or bi-weekly administration). There are several long-acting rhGH preparations in various phases of clinical development, some of which have already been approved in pediatric and adult patients with GH deficiency in Europe and Asia (61). Studies are needed in the TS population to evaluate both the efficacy and safety of such long-acting rhGH use, and to make sure treatment response will not be negatively affected by the potential for increased antigenicity of these preparations.

Conclusion

Taken together, one observes a modest gain in height with rhGH treatment in girls with TS, although treatment response varies widely between individuals. In addition to this height gain, the relatively benign safety profile and potential benefit in decreasing certain cardiovascular risk factors have supported rhGH as a standard of care treatment for girls with TS. Given the variety of TS phenotypes, age of diagnosis, and

patient/family goals, individualized treatment plans should be developed and monitored by pediatric endocrinologists so that rhGH therapy may be used to achieve optimal outcomes and improve quality of life.

Disclosure

Dr. Backeljauw is currently a consultant for Novartis/Sandoz, Novo Nordisk, and Ipsen, and receives research support from Ipsen, Opko, and Novo Nordisk.

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Growth Hormone Treatment for Prader-Willi Syndrome

Maïthé Tauber[1,2,3], MD, Gwenaelle Diene[1,4], MD, Catherine Molinas[1,2,3], CRA

[1]Centre de référence du syndrome de Prader-Willi, Hôpital des Enfants, CHU Toulouse, France, [2]Axe Pédiatrique du CIC 9302/INSERM. Hôpital des Enfants, Toulouse, France, [3]INSERM U1043, Centre de Physiopathologie de Toulouse Purpan, UPS, France, [4]INSERM UMR 1027-Université Toulouse III Hôpital Paule de Viguier, Toulouse, France

Corresponding author: Maithé Tauber, MD, Centre de référence du syndrome de Prader-Willi, Hôpital des Enfants, 330, Avenue de Grande Bretagne, TSA 70034, 31059 Toulouse Cedex 9, France, Tel: +33 534 558 551, Fax: +33 534 558 558, E-mail: tauber.mt@chu-toulouse.fr

Abstract

The European Marketing Authorization for recombinant human growth hormone (rhGH) in children with Prader-Willi syndrome was the first indication for metabolic and body composition effects in children. In the US it is indicated for short stature associated with PWS. Recombinant hGH is the first treatment for the PWS population and radically changed the care of these children by facilitating access to physicians who prescribe rhGH, mainly paediatric endocrinologists, and manage the organization of multidisciplinary care. Recombinant hGH treatment improved linear growth, body composition, and socialization not only in children but also in young adults. The pathophysiology of combined hormonal deficiencies including GH is starting to be unravelled. We now have to focus on co-morbidities that are not modified by rhGH treatment, such as feeding disorders and behaviour problems, to truly change the life of patients. The transition of care from adolescents to young adults also remains a challenge.

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***Key words**: Growth Hormone, Prader-Willi Syndrome*

Introduction

Recombinant human growth hormone (rhGH) therapy in children with Prader-Willi syndrome (PWS) was approved as an orphan drug in the United States of America in 2000 and in Europe in 2001. It was the first authorized treatment for this severe rare genetic disease and in Europe, the first non-growth related indication of rhGH targeting improvement of body composition. As written in the Consensus Guidelines published in 2013 (1) after a consensus workshop supported by the Growth Hormone Research Society in Montreal, "[rhGH] use in PWS represents a unique therapeutic challenge which includes treating individuals with disability, varied therapeutic goals that are not focused exclusively on increased height, and concerns about potential life-threatening adverse events."

Benefits of rhGH treatment have been widely documented by various centres of expertise in pediatric endocrinology and in PWS (2-4). These well established benefits mainly on height and body composition have been weighed against potential adverse events, including the rare occurrence of sudden or unexplained death that had to be documented.

Despite the difficult discussion on the benefit-risk ratio of rhGH treatment with parents prior to the initiation of treatment,

most children with PWS, particularly when seen in reference centres, are now starting rhGH during the first year of life.

This review summarizes the modern view of the disease, the story of the Marketing Authorization for rhGH treatment, the benefits and potential adverse effects of rhGH treatment, long term outcome, recent data on pathophysiology and finally the issues that remain to be adressed.

Prader-Willi Syndrome: a Disease Model

PWS is considered a complex disorder primarily characterized by impaired development of the hypothalamus that explains most of the phenotype and the natural history of the disease.PWS which is the first known example of a human disorder involving genomic imprinting documented in 1989, results from the loss of function of the paternal copy of chromosome 15 q11.2-13. Sixty % of the patients have a deletion of the paternal copy and approximately 36% of the patients have a maternal uniparental disomy for chromosome 15 (5). The remaining patients, 4%, have either an imprinting defect or a translocation or another structural alteration in chromosome 15.

The phenotype, first identified by Prader, Labhart and Willi in 1956 (6), includes neonatal hypotonia with poor oral skills and anorexia, poor social abilities, facial dysmorphia, acromicria, early onset of morbid obesity with hyperphagia and impaired satiety, endocrine dysfunction including short stature due to GH deficiency, hypothyroidism, premature adrenarche, hypogonadism (**table 1**), learning disabilities and behavioural problems with psychiatric phenotypes. Other hormonal systems have been shown to be abnormal such as ghrelin and oxytocin (7-11). This complex disease has severe consequences and difficult management issues for patients, families and care-givers (12). We recently confirmed the incidence at birth of about 1 in 20.000 (13). High rates and varied causes of morbidity (**table 2**) and mortality throughout the natural history of the disease have been reported, mainly due to respiratory problems in infancy and childhood and obesity complications later on (14-16). Early diagnosis and an integrated multidisciplinary approach have changed the phenotype of the children long term (4,17) and have a strong impact on the co-morbidities seen in adults. The story of PWS has now moved from the most frequent cause of syndromic obesity to a complex neurodevelopmental disease with severe co-morbidities (**figure 1**). Early diagnosis offers new perspectives for combined hormonal therapy possibly including oxytocin treatment with early and long-term effects that may modify the course of the disease (18).

Table 1. Endocrine dysfunctions in PWS

GHD 80%, IGF-1 low in 95%
Small for gestational age 20-25%
Hypogonadism 60%-100%
Cryptorchidism 81%, Micropenis 25%
Premature or exaggerated adrenarche 30%
Hypothyroïdism 30% to 80%
Central adrenal insufficiency 10%
Leptin ↑
Ghrelin ↑
Oxytocin ↑

Table 2. Co-morbidities in patients with PWS: a complex disease that requires comprehensive evaluation

Apneas, sleeping disorders 100% ± narcolepsy, excessive daytime sleepiness
Catatonia and epilepsia
Scoliosis 80%
Skin picking
Dysautonomia
Gastrointestinal problems may lead to severe and life threatening complications
Ophthalmologic and dental issues
Speech and language problems, oral skills and communication issues
Cognitive evaluation, learning deficits
Behaviour and psychiatric evaluation
Family and caregivers guidance
Schooling orientation

rhGH Treatment in PWS: a Success Story with Unpredictable Consequences

RhGH treatment for PWS was obtained as an orphan drug to treat short stature with or without GH deficiency (GHD) and in Europe to optimize body composition. It was a new

paradigm to use rhGH in children for a non-growth indication targeting GH effects on muscle including muscle tone, adipose tissue, and possibly cognition. This Marketing Authorization was obtained after one very smart study performed in the Nordic countries (19), accepted by both FDA and EMA, showing dramatic effects of rhGH treatment with growth acceleration and decrease of BMI and relapse after interruption of treatment. It was extraordinary to see that discordant studies about the existence or not of GHD, did not alter the decision of the agencies to allow marketing of rhGH treatment for children with PWS. **Figure 2** showed the data of this first study.

Positive Effects of rhGH Treatment in Children with PWS

Many other studies subsequently confirmed the positive effects of rhGH on growth and body composition, allowing patients to reach a normal height, normal BMI and better socialization (4,17,20,21) (**figure 3**). First results on adult height were obtained in 2007 (22) and later confirmed to be in the normal ranges (23). A poor response in respect to adult height seemed to be due to either poor pubertal height gain, possibly due to insufficient steroid hormone supplementation (22) and/or aggressive pubarche with early bone maturation (24,25).

Long Term Effects of rhGH Treatment

Interestingly, even many years after cessation of rhGH, adults who had received treatment in childhood had lower BMI and better metabolic outcome including less type 2 diabetes (26). In addition we documented long lasting positive changes in adipose tissue of young adults after discontinuation of rhGH treatment. Patients who received rhGH as children have smaller sized adipocytes than those who never received rhGH (26). In very young children fat tissue biopsies taken prior to rhGH treatment revealed that the numbers of progenitors cells were lower compared to healthy controls and that and after one year of rhGH treatment function

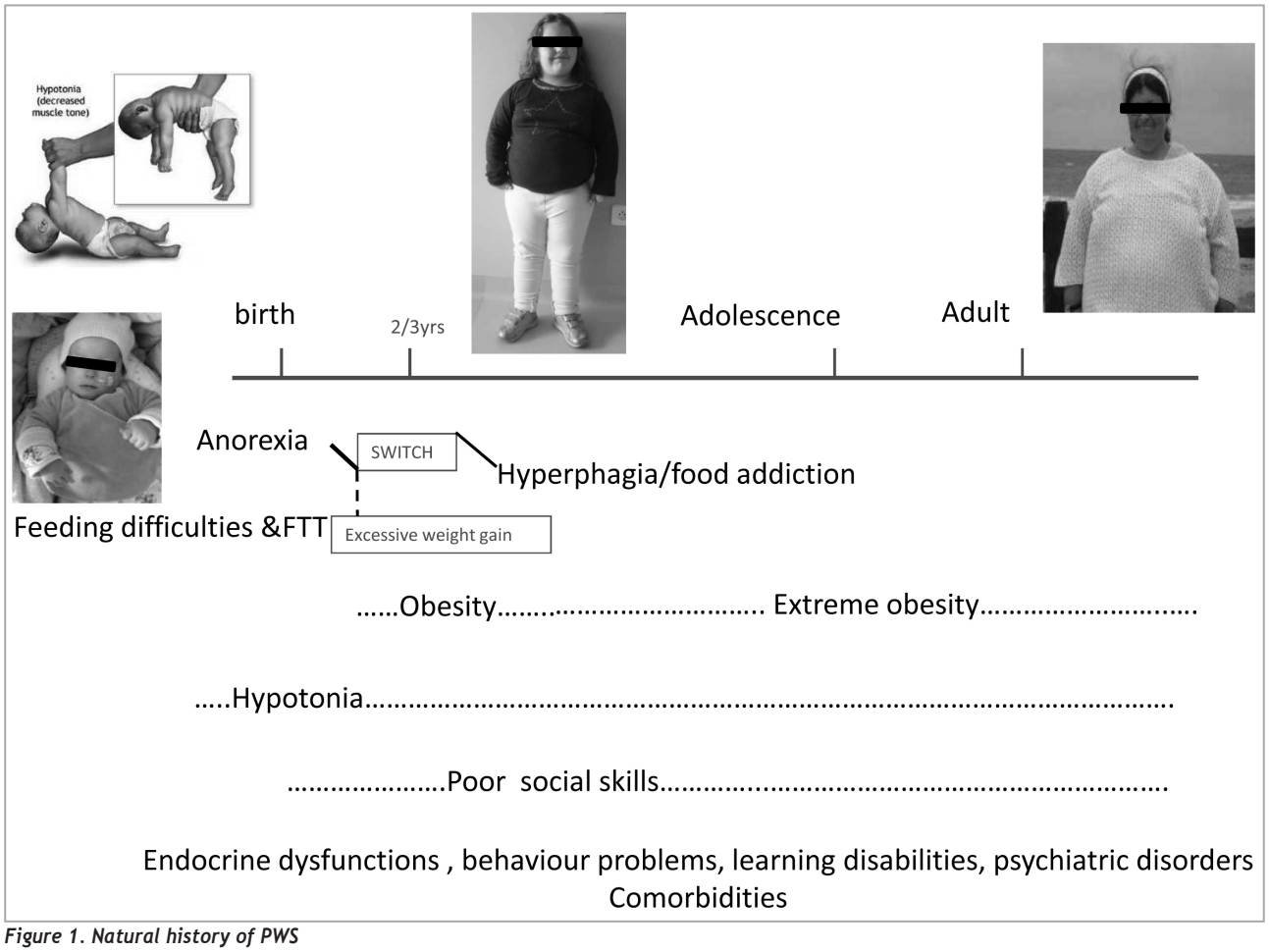

Figure 1. Natural history of PWS

and structure of adipose tissue were positively modified by GH with normalization of lipolysis and of the numbers of progenitor cells (27).

Adverse Effects

There were many publications on adverse effects of rhGH treatment in PWS that could have withdrawn the Marketing Authorization (28). Indeed sudden death of young children on rhGH treatment were reported shortly after the approval of rhGH therapy for PWS (29,30) and prompted the pharmaceutical company, Pfizer, to raise an alert on the use of rhGH in this population. This alert was subsequently removed in the absence of convincing data. We published in 2008 a review of 64 PWS deaths in infancy and childhood (14) and did not find a significant difference between treated and untreated PWS patients in the causes of deaths that are mostly (60%) due to respiratory problems, either insufficiency or infections. However, most of the deaths occurred during the first 9 months of rhGH treatment, thus suggesting a possible at-risk period and the need for holistic evaluation prior to starting treatment. Indeed longitudinal studies showed that respiratory problems may worsen after 6 weeks of rhGH treatment but that they improve thereafter during rhGH treatment (31). It is therefore essential to monitor adenotonsillar hypertrophy and IGF-1 levels. Of note, respiratory problems are also the major cause of death in adults with PWS with or without obesity (16).

In a recent review of the literature, we found no reported negative effects of rhGH treatment regarding obstructive sleep apnoea, diabetes, scoliosis and premature adrenarche, nor reported long term effects on cancer, leukemia and cerebrovascular diseases (31).

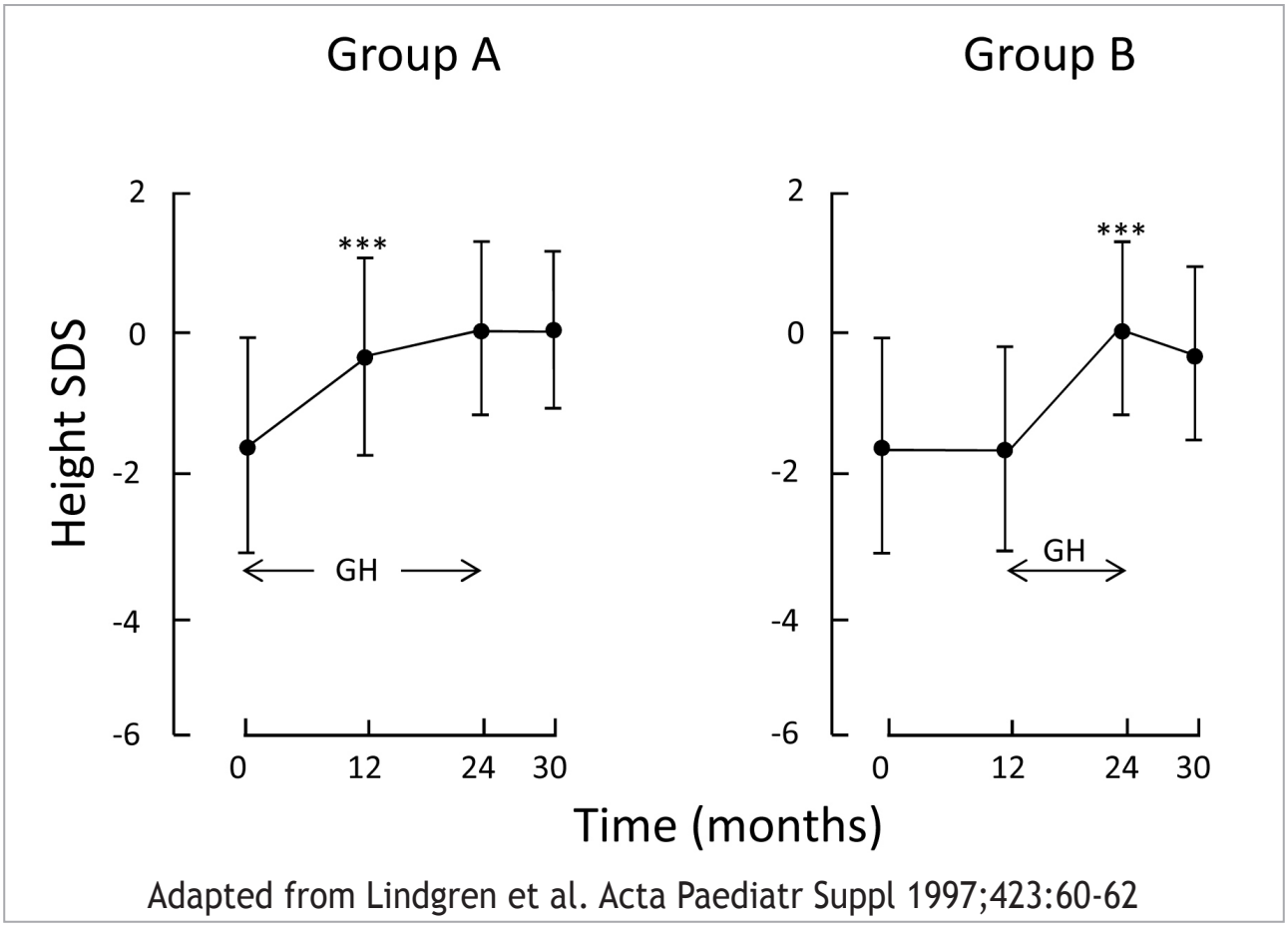

Figure 2. Height SDS during rhGH treatment in children with Prader-Willi syndrome

The rhGH-treated group (group A; n=15) was given rhGH (0.1 IU/kg/day) for the first 2 years of the study, whereas the control group (group B; n=12) remained untreated for the first year and was given rhGH (0.2 IU/kg/day) for the second year. Values are means ± SD.

******p<0.001 compared with baseline***

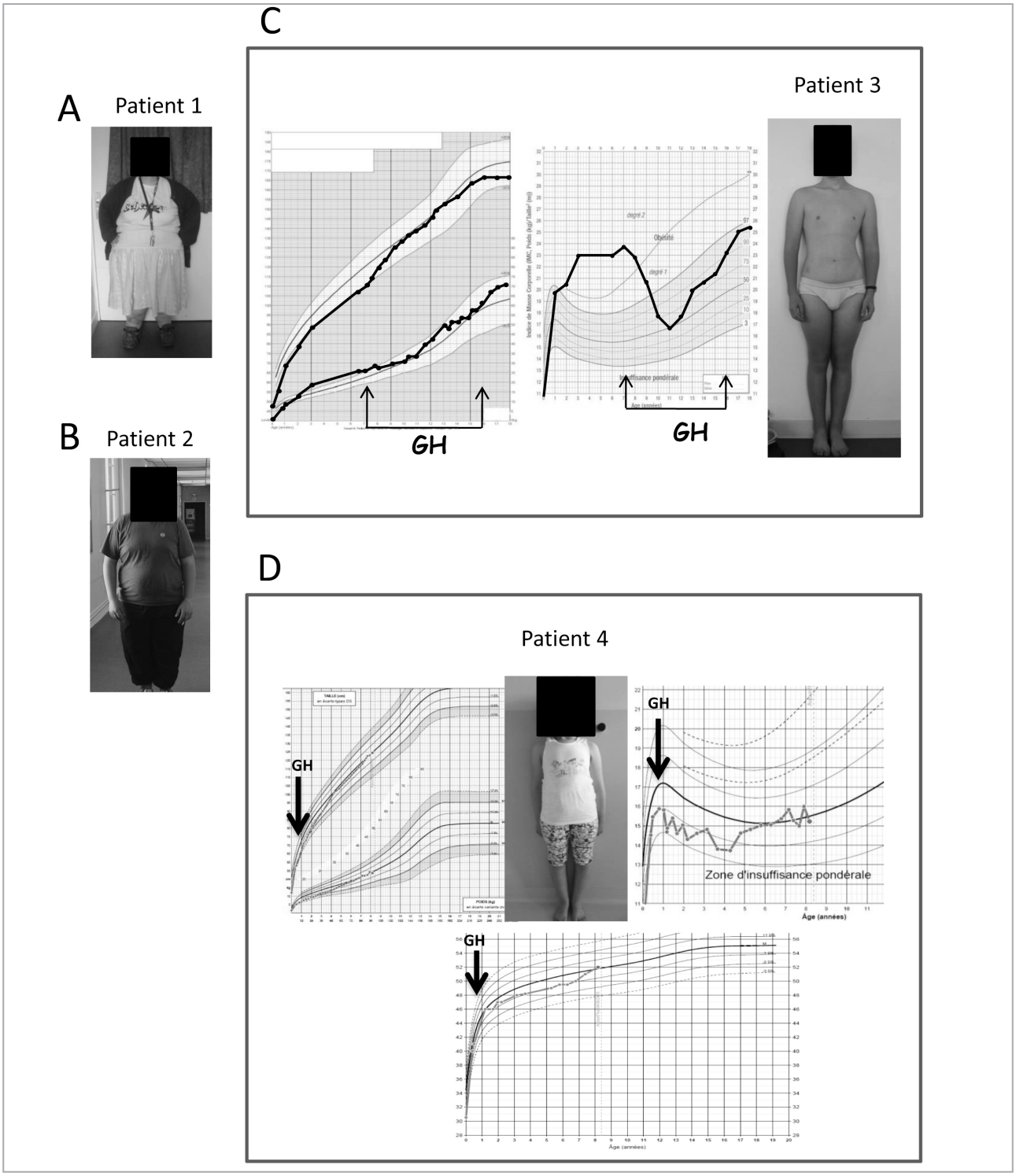

Figure 3. Effect of age at initiation of rhGH treatment

A. *Patient 1 (female) did not receive rhGH in childhood.*

B. *Patient 2 (male) started rhGH treatment at 14 years.*

C. *Patient 3 (male) started treatment at 7 years with very positive effects on growth and BMI shown in the respective charts. Stopping rhGH at completion of growth induced a subsequent increase of BMI that was controlled after resuming rhGH treatment.*

D. *Patient 4 started rhGH treatment during the first year of life with normalization of growth, weight and head circumference shown in the respective charts.*

Transition from Young Adolescence to Young Adulthood in PWS

The initial Marketing Authorization (MA) of rhGH for PWS involved only children aged from 3 to 12 years up to completion of growth. With time, increased recognition of PWS has led to diagnosis of children at younger ages, now mostly as newborns or infants. This allows children to potentially start rhGH treatment earlier than the MA, and recommendations (1) propose to initiate discussion on rhGH with parents to consider positive effects and benefit/risk ratio soon after diagnosis. On the other hand, there is no recommendation on rhGH treatment either for adults or for adolescents after completion of growth. There is only 1 publication during the transition period that showed significant negative impact on BMI after stopping rhGH treatment and its correction by resuming treatment (32). This unique study is in favour of continuing treatment regardless of the existence of GHD. Until now pharmaceutical companies who obtained the MA did not perform pivotal studies in young adults after transition, nor in adults naïve of rhGH treatment.

In France, in our reference centre, we recommend to perform a complete evaluation of all pituitary hormones with one GH stimulation test at the end of growth and resume rhGH in agreement with the patient and his/her parents if the GH peak is below 6 ng/ml, the cut-off accepted in adolescents to define GHD (**figure 4**). Treatment is stopped if the patient wants to stop and/or if testing excludes GHD. Subsequent follow-up every 6 months is continued for all adolescents and in cases of worsening BMI with or without excessive fatigue and in agreement with the patient and his/her family, we propose to resume rhGH treatment. This follow-up requires organized transition between paediatric centres and the few centres with expertise in adults with PWS. Indeed, we have recently shown that good paediatric care and transition is key for the outcome of young adults (33).

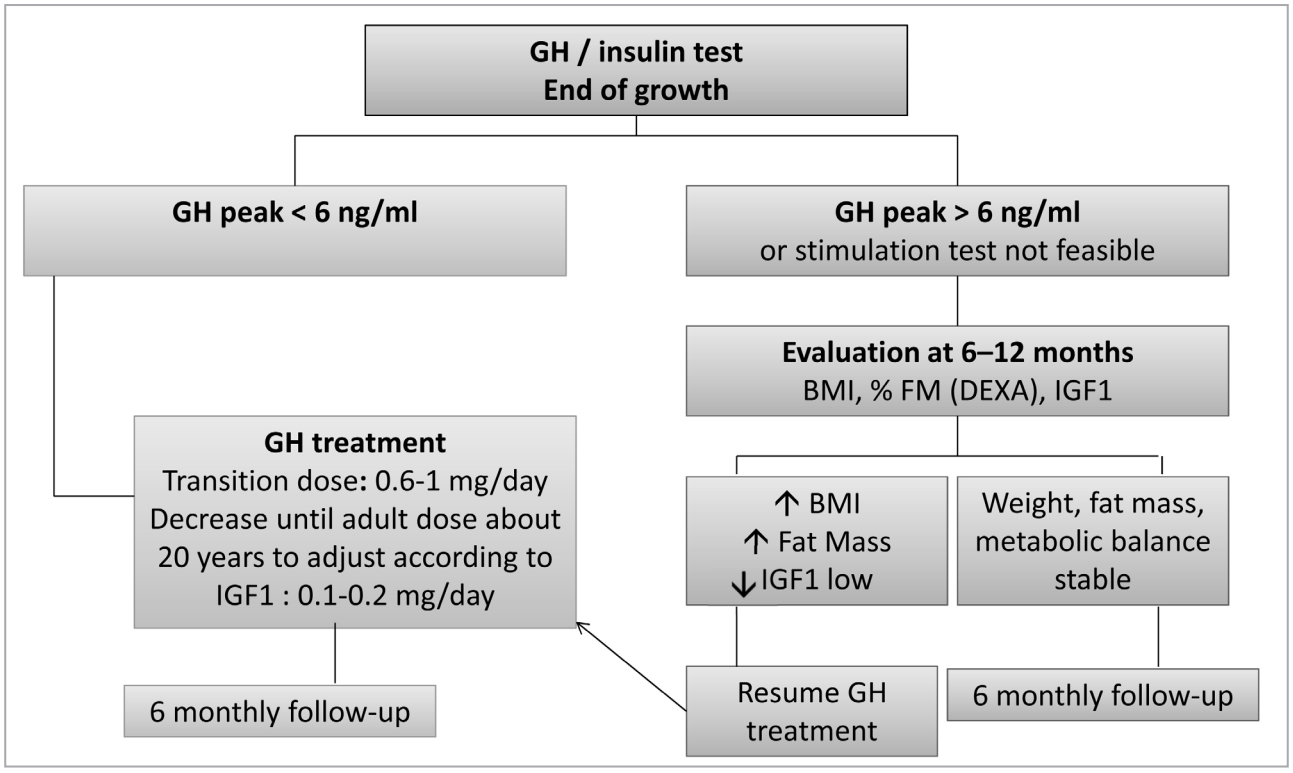

Figure 4. Transition and growth hormone treatment

Integrated View of PWS: Pathophysiology of Endocrine Dysfunction

We recently participated in a collaborative work using induced pluripotent stem (iPS) cells from patients with PWS (34) and a very unique patient carrying the smallest deletion of the SNORD116 gene cluster displaying the key features of PWS we published in 2015 (35). Hypothalamic neurons obtained after redifferentiation of iPS cells had a decreased level of the transcription factor, NHLH2 that suppresses transcription of the gene PCSK1 coding for the Proconvertase 1 (PC1). This observation was made in cells obtained from patients with PWS as well as from the unique patient with the smallest deletion, suggesting that the deficit is due to deletion of the SNORD 116 gene cluster. PC1 is needed for the processing of prohormones to mature/active hormones It is thus suggested that the hypothalamic PC1 deficit in PWS may impair endocrine function.

Recently, a hypothalamus-targeted mutation of the SNORD116 locus in mice was shown to recapitulate in some but not all animals the entire natural history of PWS from failure to thrive to hyperphagia and obesity (36).

Other studies have shown that one of the maternally imprinted genes in the PWS region, the MAGEL 2 gene, was involved in autism (37), arthrogryposis muscle dysfunction (38,39) and development of the hypothalamus. It is clear that the complex chromosomal region involved in PWS is crucial for both the development and the function of the hypothalamus (**figure 5**).

Conclusion

rhGH has been used for almost 20 years in treating patients with PWS with very positive effects that have dramatically changed the lives of the children, their families and care-givers. Challenges remain to treat adolescents and young adults during the transition period, to help prevent loss to follow-up and to document the benefit/risk ratio in adults. In addition, we still have to unravel the main pathophysiologic mechanisms behind this complex disease in order to improve comprehensive treatment and management. Indeed, many drugs are now being investigated in PWS targeting feeding problems, obesity and behaviour. Early diagnosis offers an opportunity to implement early treatment that may cure the first nutritional phases and modify the course of the disease. PWS is a disease model for studying hypothalamic development, early diagnosis and interventions, feeding disorders and behaviour troubles with very recent and interesting therapeutic perspectives.

Disclosure

MT received honoraria from Pfizer, NovoNordisk, Ipsen, Lilly, for advisory board meetings and symposia and received funding for clinical and basic research projects from Pfizer, Ipsen and Novo Nordisk.

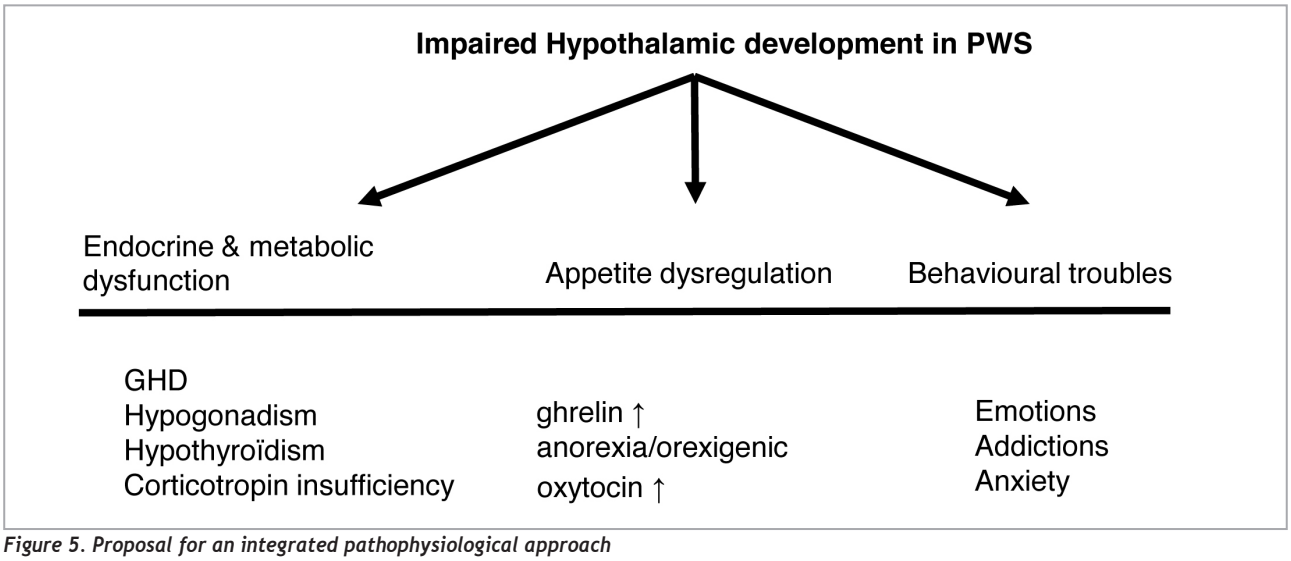

Figure 5. Proposal for an integrated pathophysiological approach

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Growth Hormone Treatment for Patients with Noonan Syndrome

Ximena Gaete[1,2], MD, Fernando Rodríguez[1], PhD, Fernando Cassorla[1], MD

[1]Institute of Maternal and Child Research, University of Chile, Santiago, Chile, [2]Clinica Alemana, Santiago, Chile

Corresponding author: Fernando Cassorla, MD, Institute of Maternal and Child Research, School of Medicine, University of Chile, Santiago, Chile, Tel: 562-29770860, Fax: 562-24247240, E-mail: fcassorl@med.uchile.cl

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Abstract

Noonan syndrome (NS) is a genetic disorder, which can present clinically with a variable phenotype. Proportional post natal short stature is a common manifestation of NS, with the majority of affected patients having an adult height below the third percentile. Some investigators have reported minor abnormalities in GH secretion and/or action, suggesting that recombinant growth hormone (rhGH) therapy may be useful for the treatment of their short stature. Our review of the literature regarding rhGH therapy in children with NS indicates that this therapy improves height velocity, but relatively few controlled clinical trials reporting adult height are available. rhGH treatment does not appear to be associated with adverse effects in these patients, but data on the possible development of malignancy during treatment are somewhat limited. Therefore, we believe that there is a need for large controlled clinical trials in patients with this condition, in order to accurately assess the effects of rhGH therapy over adult height.

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Key words: Noonan Syndrome, Growth hormone, Adult height

Introduction and Clinical Features

Noonan syndrome (NS) is a genetic, multisystemic disorder which can present clinically with a variable phenotype. The estimated prevalence of this syndrome has been reported between 1 in 1000 and 1 in 2500 live births (1). NS may occur on a sporadic basis, as a de novo mutation or following an autosomic dominant inheritance. The main clinical characteristics of the syndrome consist of short stature, cardiovascular abnormalities (pulmonary valve stenosis, left ventricular hypertrophy), cryptorchidism, special facial features (hypertelorism, down slanting palpebral fissures, low posterior rotated ears, webbed neck, chest and spinal deformities), and learning disabilities with mild mental retardation (2,3). Proportional post-natal short stature is a common manifestation of NS, occurring in more than 80% of patients, with the majority of affected patients having a height below the third percentile (4).

Genetic Aspects

The first gene directly associated with NS was PTPN11 (5). This gene encodes the phosphatase SHP-2 which regulates several different signaling pathways (6). In 2004 Fragale showed that three NS causative PTPN11 mutations increase SHP-2 phosphatase activity and prolong the intracellular signal through the RAS/mitogen-activating protein kinase pathway (RAS/MAPK pathway) (7). This pathway is activated by growth factor binding to receptor tyrosine kinases which generate docking sites at cytoplasmic tyrosine residues for adaptor molecules (e.g. GRB2). This adaptor recruits and activates the guanosin exchange factor SOS1 which induces GDP by a GTP change at the nucleotide binding site for the RAS protein (KRAS, NRAS, HRAS). In its GTP bound conformation (activated), the RAS protein can transmit signals through

different routes such as RAS-mediated mitogen-activated kinase (MAPK). In this pathway GTP-RAS interacts and activates RAF1 and/or BRAF, and these serine/threonine-protein kinases phosphorylate MEK1 and/or MEK2, which in turn phosphorylates and activates EKR1 and/or ERK2. Several studies performed between 2006 and 2013 disclosed a strong association between missense substitutions in other components of the RAS/MAPK pathways (SOS1, KRAS, NRAS, RAF1, BRAF and RIT1) and Noonan syndrome, with all mutations activating the pathway (8). The current distribution of RAS/MAPK pathway gene mutations in Noonan syndrome is approximately PTPN11 (~50%), SOS1 (11%), RAF1 (5%), RIT1 (~5%), BRAF (~2%), KRAS (~1.5%) and NRAS (0.2%).

Growth in Noonan Syndrome

In general the birth weight and birth length of these patients is normal or only slightly subnormal (9). Subsequently, a loss in height SDS of 1.0 -1.5 SD occurs during the first year of life, and mean height follows the 3rd percentile from 2 until 12 years in males, and 10 years in females. There is an additional decline in growth velocity caused by late puberty in most of these patients, and their limited pubertal growth spurt is characterized by a relatively low peak height velocity (10).

Causes of Short Stature in NS

The cause of short stature in this condition remains poorly understood. Different potential mechanisms have been reported, including GH deficiency (11), neurosecretory dysfunction (12) or GH resistance (13). Noordam *et al*, found normal levels of IGF-I and IGFBP-3 and normal responses to GH stimulation tests, but observed a low mean GH concentration in the 12-hour overnight GH profile. These relatively minor abnormalities in GH secretion in patients with NS are unlikely to be the major cause of their growth retardation (14), but suggest that GH therapy may play a role in the treatment of their short stature.

Adult Height

Noonan *et al*, reported their findings in 73 adults over 21 years old (29 male and 44 female), and observed that 31% of males had adult heights in the normal range, but the rest had a adult height of 167 cm or less. In the case of the females, 32% had a adult height of 155 cm or more, whereas the remainder had an adult height between 132 and 152 cm (15). Ranke *et al* (10) found an adult mean height of 152.7 cm for females and 162.5 cm for males in 33 individuals with NS. Wit *et al*, (16) published similar data in 28 adults with NS, and observed a mean adult height of 161 cm in males and 150.5 cm in females. In addition, Shaw *et al* (17) published data from the UK documenting a mean adult height of 167.4 cm for males and 152.7 cm for females with NS.

Growth Hormone Treatment in NS

Growth hormone treatment (rhGH) has been shown to increase the growth rate in patients with NS, but the benefit of long term therapy over adult height is still subject of some debate. The overall experience with long-term rhGH therapy in NS is rather limited, so NS patients who have received rhGH treatment until adult height represent a relatively narrow segment of the patients studied. The US Food and Drug Administration approved treatment of NS with rhGH in 2007 recommending doses up to 0.066 mg/kg/day, but this indication has not been approved by the European Medicine Agency.

Most of the evidence regarding rhGH therapy in patients with NS originates from observational uncontrolled studies in relatively small numbers of subjects. Thus, no controlled clinical trials reporting adult height in these patients have been published, but a few longitudinal prospective trials and some longitudinal retrospective studies based on post marketing studies have been published (18). The available data are difficult to compare due to the heterogeneous treatment protocols employed, as well as the different cohort selection criteria. In addition, the age at onset of GH treatment and the rhGH doses employed are quite variable, and tend to be higher in case studies (26-66 ug/kg/day) compared with observational studies (33-43 ug/kg/day). An interesting fact that emerges from this literature is that the NS patients who were treated with rhGH were shorter than their NS peers at baseline.

Several short-term studies with less than one year of rhGH therapy for NS patients have reported an increase in height velocity and height SDS during this period (19-21), as described for other clinical conditions which are associated with short stature. In addition, Ziklar *et al*, reported the effect of rhGH treatment during three years in a group of 47 patients with NS. In this group, the height standard deviation score (HSDS) increased from -3.62±1.14 to -2.85±0.96, which was significantly different compared with the patients who did not receive rhGH therapy (22).

More prolonged studies, such as those published by MacFarlane (23) and Lee (24) also reported an acceleration of growth velocity and increase in height SDS during rhGH therapy in patients with NS. MacFarlane, in the only controlled trial reporting data from 31 children (23 treated and 8 untreated), showed that over 3 years the treated group gained an average of 3.3 cm more than the untreated group. In addition, Romano *et al* (25) evaluated the response to rhGH therapy in 150 children with NS by analyzing the growth data from children with NS who were enrolled in the National Cooperative

Growth Study, which were compared with children treated with rhGH for isolated growth hormone deficiency and Turner Syndrome. Children with NS monitored for at least 4 years had a significant increase in height SD scores, and their growth velocity was greater than girls with Turner syndrome treated for the same period.

Lee *et al* (24) compared the responses to rhGH therapy in patients with Turner Syndrome (TS) and NS which were enrolled in the Nordinet International Outcome Study (IOS), and demonstrated that both groups of patients achieved a similar response to 4 years of rhGH therapy. In NS patients and TS patients 4-year adjusted ΔHSDS were +1.14 ± 0.13 and +1.03 ± 0.04, respectively. Based on untreated, disease-specific references, the four year adjusted ΔHSDS for NS and TS were +1.48 ± 0.10 and +1.79 ± 0.04 ($p < 0.0001$) respectively, with ΔHSDS being higher with a younger baseline age in NS.

Adult Height /Near-Adult Height (Table 1)

Adult or near-adult height data have been reported from three main (26-28) and two small studies (29,30). As observed in the **Table 1**, age at start of treatment and duration of rhGH varied widely between these studies, whereas mean heights at the start of treatment were similar. These studies show a relatively large variation in the height gain observed after rhGH therapy (0.6–2.0 SDS), with the best results reported in the patients who were younger at the start of the treatment. More recent data suggest that an additional spontaneous height gain of 1.0 SDS may occur during the second decade of life in girls, with a further gain of 0.57 SDS which may occur at the start of the third decade in boys (31). Therefore, the potential positive effects of a late pubertal growth spurt should be considered in the projections of adult height for short children with NS.

The study of Raaijmakers, is based on a large cohort of patients with NS from the KIGS database. Near adult height was reached in 24 patients after at least 4 years of treatment. The median age at the start of treatment was 10.2 years and the median duration of rhGH was 7.6 years. Median gain in height SDS was 0.61 according to Tanner standards and 0.97 SDS according to Noonan standards. No serious adverse events related to GH were observed in this study (28). In addition, Noordam *et al*, published adult height data in 29 patients with NS. Their mean adult height SDS was –1.5 and +1.2 according to National and Noonan standards, respectively. The mean adult height for boys was 1.71 m and 1.57 m for girls. This indicates a gain in adult height of 1.3 SDS which corresponds to approximately 9.5 cm. Linear regression analysis showed that age at start of puberty made the only statistically significant contribution to the gain in adult height (26). Osio *et al*, reported adult height in 18 children with NS who were treated with rhGH in an uncontrolled study during a mean period of 7.5 years. This author reported an increase in adult height from −2.9 to −1.2 SDS after completion of rhGH treatment. This was equivalent to an increase of 10.3 cm compared with predicted adult height. In addition, he observed a further mean height gain of 0.9 SDS in males and 0.5 SDS in females during late puberty, reaching their adult height at a mean age of 19.5 years (range 17–21 years) (27). Other studies of rhGH therapy for NS patients, have shown that the overall height gain of patients is limited, possibly due to the fact that therapy usually starts at the relatively late age of 10 years, when the height of these patients is quite low, at approximately −3.0 SDS (29, 30). In addition, the limited response to therapy may reflect the variability in rhGH doses employed, and the fact that some of these patients may exhibit some evidence of impaired GH sensitivity (14).

Table 1: Published adult height data in patients with Noonan syndrome treated with rhGH

	Kirk *et al* (30)	Osio (27)	Raaijmakers (28)	Noordam(26)	Romano (25)
Patients with adult height, n	10	18	24	29	65
Data source	KIGS UK	Random study	KIGS world	Random study	NCGS observational
Age at start, years	12.1 (8-15)	8.2 (3-14)	10.2	11.0 (6-18)	11.6
Height,SDS	-3.1	-2.9 (-4 to -2)	-3.3	-2.8 (-4 to-2)	-3.5 (1.0)
rhGh dose, mg/kg/day	0.035	0.033/0.066	0.035	0.05	0.05
Duration of therapy, years	5.3 (2-8)	7.5 (4-12)	7.6	6.4 (3-10)	5.6 (2.6)
Height gain , SDS (according Tanner)	0.6 (-0.2-1.0)	1.7 (0.4-0.3)	1.0	1.3 (-0.6 to 2.4)	1.2 boys, 1.5 girls
Height gain , SDS (according Noonan)	0.8	1.7 (0.5-3.1)	0.6	1.3 (-0.2 to 2.7)	0.7 boys, 0.3 female

Adapted from Dahlgren (38)

Variables Correlated with Increased Growth

The duration of rhGH therapy before puberty and the height SDS at puberty are important contributors to near adult height in patients with NS. This suggests that growth optimization may be possible with earlier initiation and longer duration of rhGH therapy in these patients. In addition, a delay in the onset of puberty also appears to have a positive effect on the treatment response (26).

No significant correlation with rhGH dose nor with gender, however, has been observed in most of these studies (24). Whether the clinical phenotype is moderate or severe does not appear to be a predictor of the response to rhGH therapy. Both phenotypes respond similarly to rhGH treatment despite a significantly higher mean GH level in the severe phenotype (32). In some short-term studies there is a correlation between the growth response and the genotype (with a smaller growth response when the patient carries the PTPN11 mutation), but this finding has not been confirmed by other authors who have not observed any differences in height gain, height velocity SDS, adult height and/or serum IGF-1 in NS patients with and without PTPN11 mutations (26,33,34).

Safety of rhGH Therapy and Quality of Life

None of the published studies has reported serious adverse effects during rhGH therapy, in particular regarding potential effects over the progression of ventricular wall thickness (35). The limited data available regarding this issue warrants caution, however, so cardiovascular function should be monitored carefully during rhGH treatment in these patients. In addition, patients with NS have a higher risk for leukemia and certain solid tumors than the general population. In patients with NS carrying the PTPN11 mutation, which is the most common cause of NS, the cumulative risk of developing cancer has been estimated to be 3.5-fold higher than in the general population (36).

Among the reported cases, Moos *et al*. (37) reported a large subcutaneous infiltrating atypical granular cell tumor on the left forearm in a child with NS treated with rhGH. Recurrence of a previously diagnosed maxillary gland giant cell granuloma has been reported in one rhGH-treated patient (25), whereas a lymphoma was reported in another patient three years after starting rhGH treatment. In the latter patient, rhGH treatment was restarted after the remission of the lymphoma (27). Recombinant human GH treatment may also result in the acceleration of bone maturation, probably due to the normalization of their retarded bone age, which is common at onset of therapy in patients with NS. Other parameters, such as BMI and glucose metabolism have remained unchanged during rhGh therapy (24).

Conclusions

In conclusion, rhGH therapy increases height velocity in patients with NS, but there is less information regarding the potential long-term benefit over adult height. Therefore, we believe that there is a need for large controlled clinical trials in patients with this condition, in order to accurately assess the effects of rhGH therapy over adult height. In addition, given the complexity of this disorder, in terms of the different underlying molecular genetic defects, the high prevalence of cardiac defects and the possible risk of malignancy, it is very important to evaluate these patients at regular intervals, particularly during and following treatment with rhGH.

Disclosure

The authors declare no conflict of interest.

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Growth Hormone Treatment for Short Children Born Small for Gestational Age

Adriane de Andre Cardoso-Demartini[1], MD, PhD, Alexsandra C. Malaquias[2], MD, PhD, Margaret Cristina da Silva Boguszewski[3], MD, PhD

[1]Pediatric Endocrinology Unit, Department of Pediatrics, Federal University of Paraná, Curitiba, Brazil, [2]Division of Pediatric Endocrinology, Department of Pediatrics, Faculdade de Ciencias Medicas da Santa Casa de Sao Paulo and the Genetics Unit (LIM25), Faculdade de Medicina da Universidade de Sao Paulo, São Paulo, Brazil, [3]Department of Pediatrics, Federal University of Paraná, Curitiba, Brazil

Corresponding author: Margaret Cristina da Silva Boguszewski, MD, PhD, Department of Pediatrics, Federal University of Paraná, Curitiba, Brazil, Rua General Carneiro, 181 (14º andar) - Curitiba, PR, Brazil, CEP: 80060-900, Tel: +55-41-3360-7994, E-mail: margabogus@uol.com.br

Abstract

Despite the difficulty to define born small for gestational age (SGA), being SGA has been associated with a higher risk of short stature, early-onset and rapid progression of puberty, neurocognitive dysfunctions, alterations in body composition, bone density, glucose and lipid metabolism and increased risk for cardiovascular diseases later in life. The majority of children born SGA experience spontaneous catch-up growth during the first years of life. For those who remain with short stature, treatment with recombinant human growth hormone (rhGH) may be initiated, preferably after 2-4 years of age. Response to treatment is variable. However, the benefits of rhGH go beyond increase in stature as the therapy may also improve body composition. In this review we will cover the indication and effects of GH therapy in short children born SGA.

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Introduction

At the beginning of the 20th century, Ballantyne observed a wasting condition of newborn and, later, Runge proposed it could be due to the impairment of the nutrient supply to the fetus. Their names were given to Ballantyne-Runge Syndrome, a condition in newborns that is now known as intrauterine growth retardation (IUGR) or small for gestational age (SGA). Nevertheless, these terms should not be used as synonyms: SGA refers to body size at birth (a low weight and/or length for a known gestational age) and IUGR refers to reduction of the growth velocity documented by at least two fetal measurements (1).

The definition of SGA is not precise and multiple criteria have been used, the majority of which relied on birthweight alone. Besides, accurate gestational dating is necessary. In 1995, the World Health Organization (WHO) recommended the definition of SGA as birthweight less than the 10th percentile for gestational age. This definition was chosen due to the increased perinatal and neonatal risks of children born SGA compared to newborns with appropriate size (2), definition previously adopted by Battaglia and Lubchenco (3). The consensus of the Pediatric Endocrinology Societies (1,4)

recommended that SGA should be defined as birth weight and/or birth length equal to or less than -2 SD for sex and gestational age, as previously suggested by Usher and McLean (5), in order to have a more homogeneous group when evaluating a child born SGA. Depending on the definition used, different results are obtained. The incidence of spontaneous catch-up growth (CUG) until 9-12 months of life was reported to be 86% when SGA was defined as birth weight below -2 SD and 79% when the 5th percentile was used (6). Additionally to this definition, SGA newborns can be sub-divided into SGA due to weight, to length or to both, all with different growth prognoses (1). Another issue with the definition of infants born SGA is the reference population. When a national reference is not available, the reference recommended by the WHO (7) has been used. Children born prematurely (before 37 weeks of gestational age) should be evaluated separately, and specific references for preterm must be used if available. Recently, a study showed that 24% of the preterm newborns who were considered SGA according to the Intergrowth-21st standards were considered appropriate for gestational age (AGA) according to the Fenton preterm growth charts (8). Customized growth charts are also proposed to adjust for physiological variation of body size in order to identify babies who are constitutionally or pathologically small. The characteristics of the mother, taking into account ethnic origin, height, weight, and parity, increases confidence in growth assessment (9). The estimated prevalence of SGA in term infants varies in several countries and regions: 2.3% in the United States (10), 5.5% in Sweden (11), 3.4% in Japan (12), 12.5% in Latin America (13) and 44.5% in South Asia (13). In children born preterm, especially below 34 weeks, the prevalence of SGA is higher (12-14).

Children born SGA have a higher risk of short stature than children born AGA. Most children born SGA show spontaneous CUG to weight and height above -2 SDS during the first 2 years of life (15,16), the majority during the first 2-3 months (17,18). Children born preterm and SGA may catch-up later (19). About 10-15% remain short and the reason for this insufficient CUG might be related to abnormalities in growth hormone (GH) secretion and/or reduced insulin-like growth factor I (IGF-I) levels (16,20), although most of the SGA children are not GH deficient. The lack of spontaneous catch-up in length within the four months of life can be used to early identify SGA children with higher risk of short stature at 5 years (21). These children will reach an adult height below the normal range for the population and/or their target height and they account for approximately 20% of all cases of adult short stature (22). The relative risk of short stature during adult life is 7.1 for those born SGA due to length and 5.2 for those born SGA due to weight (18).

Human GH (hGH) was first administered to non GH-deficient children with short stature secondary to IUGR in the early 1970s (23-25). rhGH has been used since 1986 and several studies demonstrate that the majority of short children born SGA show an increase in growth velocity during therapy and, if left untreated, would probably remain short in adult life as recently reviewed (26). It is known that some chronic adult diseases originate in critical phases of fetal development (27). Treatment with rhGH induces catch-up growth and increases adult height, but it also may modify appetite and body composition, and potentially may reduce risk factors for cardiovascular diseases later in life (28). This review will discuss the indications, safety and effects of rhGH therapy in short children born SGA on longitudinal growth and body composition, and its long-term metabolic consequences.

Growth Hormone Treatment

Treatment with rhGH has been indicated for children born SGA who failed to present a spontaneous CUG, defined as growth velocity (cm/yr) greater than the median for chronological age and gender to achieve a height above -2 SD. Premature SGA infants may take a longer time to recover. Approximately 3 to 10% of the SGA children fall into this definition (1). In 2001 the US Food and Drug Administration (FDA) approved the use of rhGH for children born SGA with height < -2 SDS starting from age 2 years at a recommended dose up to 0.068 mg/kg/day. In 2003, the European Agency for the Evaluation of Medical Products (EMEA) also approved rhGH for children born SGA with height below -2.5 SDS by the age of 4 or older, with the recommended dose of 0.035 mg/kg/day until adult height. In 2008, the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan approved rhGH treatment for short children born SGA with height < -2.5 SDS starting from 3 years at a starting dose of 0.033 mg/kg/day up to 0.067 mg/kg/day (29). Statements from international societies of pediatric endocrinology recommended rhGH treatment in a dose of 0.035 to 0.070 mg/kg/day for SGA children with height below -2.5 SDS at the age of 2 years or with height below -2 SDS at the age of 4 years (1,4). It is noteworthy that the term SGA is not a definitive diagnosis. Before starting rhGH therapy, efforts should be made in order to define the etiology of short stature in SGA children. Endocrine disorders, chronic diseases, use of medications that impair growth (e.g. corticosteroids), and IUGR due to specific syndromes should be ruled out. Bloom syndrome, Fanconi anemia, and Diamond-Blackfan anemia are examples of syndromes with prenatal growth retardation in which rhGH treatment is not indicated due to the inherent increased risk for malignancy (30).

The primary goal of rhGH treatment is to increase prepubertal growth velocity and achieve a normal height during childhood. The ultimate objective is to reach an adult height within target height. Determinants of the response to rhGH treatment over the first 2–3 yr show a negative correlation to age and

height SDS at the start of therapy, and a positive correlation to rhGH dose and mid-parental height (1). Several clinical studies/case reports have described an increase in growth velocity in short children born SGA treated with rhGH during a short follow-up period. However, few of these studies are randomized controlled trials and fit the Endocrine Society criteria for high quality of evidence (31). Recently, a systematic review of four randomized controlled trials (32-35) evaluated the impact of rhGH treatment in short SGA children (36). All of them were considered of moderate to high quality according to the Endocrine Society grading (**table 1**). Children treated with hGH reached a mean adult height exceeding untreated children by 0.9 SD, (approximately 6 cm) after ~8 years of rhGH therapy, in agreement with previously reported meta-analysis (37). As discussed before, children born SGA comprise a heterogeneous group and syndromic SGA children might present different outcomes. Genetic factors influencing small size at birth encompass pathogenic variations involving GH/IGF-I axis genes (GHR, GHRHR, STAT5B, IGF1, IGF2, IGFALS, and IGF1R), chromosomal abnormalities, uniparental disomy (UPD) and imprinting effects, among others. Growth improvement was shown in patients with IGF1 and IGF1R variants and children with Silver-Russell syndrome, but the effectiveness of long-term therapy in these situations remains under investigation (6,7).

Cumulative safety data from observational studies supported by manufacturers of rhGH have involved more than 120,000 children treated with rhGH and endorsed a safety profile since rhGH was approved for use in children with GH deficiency by the FDA in 1985. In general, long-term rhGH therapy for SGA children is safe and well tolerated (37). However, some concern remains about long-term safety of rhGH treatment with doses higher than 0.050 mg/kg/day (9). Although rhGH treatment was not associated with an increased risk of malignancy in children without previous risk factors for malignancy, it has been recommended that IGF-I concentrations shoud be monitored and maintained in the normal range. Increased IGF-I levels were reversed entirely after discontinuing rhGH (1,4).

Treatment with Growth Hormone and Puberty

Studies of puberty in children born SGA have shown conflicting results. Precocious adrenarche and pubarche, earlier onset of pubertal development and earlier peak height velocity were reported (38-40), but also no difference in age of pubertal onset and spurt between children born SGA and AGA. Height at onset of puberty was reported to be less than expected (41,42). Normal age range, earlier and no differences in age of menarche (38-40,42) between girls born SGA and those born AGA (43,44) were also reported. It has been suggested that children born SGA may present accelerated epiphyseal fusion (45) with bone age equal or greater than chronological age at pubertal onset during rhGH treatment (46-48). Boonstra *et al*. showed that rhGH

Table 1: Randomized trials of rhGH therapy in short children born SGA (case-controls) showing adult height

Author (year)	n	rhGH dose (µg/kg/day)	Mean age at start	Mean therapy duration (years)	Mean AH or NAH SDS*	Height gain (SDS)	Target / mid-parental height SDS*
van Pareren et al. (2003) (31)	28	33	7.9	7.9	-1.1	1.8	-0.9
	26	67	8.2	7.5	-0.9	2.1	-0.5
	15	None	7.8	-	-2.3	0.3	-1.1
Carel et al. (2003) (32)	102	67	12.7	2.7	-2.1	1.1	-0.9
	47	None	12.8	-	-2.7	0.5	-1.2
Dahlgren&Wikland (2005) ¥ (33)	36	33	8.9	8.5	-1.2	1.9	-1.2
	41	33	12.3	5.5	-1.6	0.9	-1.2
	34	None	8.3	-	-2.0	0.2	-1.1
van Dijk et al. (2007) (34)	37	33 and 66	8.5	7.3	-1.4	1.5	-
	25	None	7.8	-	-2.6	0	-

** national standards; ¥ 2 groups divided according <2 prepubertal years (36) or >2 prepubertal years (41) with rhGH treatment*

Abbreviations: SGA, Small for Gestational Age; rhGH, Recombinant Human Growth Hormone; SDS, Standard Deviation Score; AH, Adult Height; NAH, Near Adult Height.

treatment did not affect pubertal onset and progression in children born SGA (47), however a less intense pubertal height gain compared to AGA pairs was reported (25.5 cm in SGA boys and 15.3 in SGA girls compared with 28 cm in AGA boys and 25 cm in AGA girls, P < 0.001) (46). High dose of rhGH was shown to induce acceleration of bone age and onset of puberty in children with idiopathic short stature (ISS) (49). Taken all together, a closer follow-up of height gain and pubertal progression is advised in this group of children, especially during rhGH treatment.

Although the recommendation is to start treatment with rhGH between 2 and 4 years of age, many children start treatment near or at the beginning of puberty. For these children, the combination with gonadotropin-releasing hormone analogues (GnRHa) may be beneficial, with the possibility to delay epiphyseal maturation and to prolong time for growth. The combined treatment is controversial, and adult height in short children with normal pubertal timing might not be improved (50,51). Kamp *et al*. showed a significant reduction of growth velocity during the second and third years of treatment with delay of bone maturation in a small group of pubertal children with ISS or born SGA treated with the combined drugs (triptorelin 3.75 mg/month, rhGH 0.05 mg/kg/day). They reported a significant mean gain in predicted adult height (PAH) of 8 cm in girls (11.1 cm in SGA girls) and 10.4 cm in boys (6.5 cm in SGA boys) and changes in body proportion with longer legs compared to untreated children (52). However, they also reported a median adult height significantly lower than target height in both groups with no difference in adult height between those treated with the GnRHa and rhGH and the untreated ones. Even though a modest but significant height gain was observed in the treated group (4.4 vs. -0,5 cm, P < 0.05), they concluded that the increase in PAH was lost during follow-up and it was overestimated, and that the combined treatment should not be considered routine for short children SGA born or with ISS (53).

Recently Lem *et al*. (54) and van der Steen *et al*. (48) showed that the combined therapy could be beneficial for children with stature below 140 cm at the onset of puberty and PAH below -2.5 SDS. The combined therapy in these children resulted in adult height similar to subjects whose puberty started with height above 140 cm and treated with rhGH only (48,54), although they observed a shorter pubertal duration after GnRHa discontinuation probably due to growth plate senescence. In the Dutch study, height gain during combined therapy was more expressive with higher GH doses (0.067 vs. 0.033 mg/kg/day) only in boys (12.7 vs. 15.2 cm, P = 0.015), though height gain after GnRHa cessation to adult height was similar with both doses (54). Adult height was 0.6 SDS higher in the double dose group (P = 0.002), showing the potential benefit of higher rhGH doses during the period of GnRHa use (54), although another study found that height before onset of puberty had a stronger association with pubertal height gain than the dose of rhGH, reinforcing the need to start rhGH treatement before puberty (55). When treatment with GnRHa is considered an option, more than three years of treatment might be necessary (56).

Effects of rhGH Therapy Beyond Longitudinal Growth

Table 2 resumes the short- and long-term effects of rhGH treatment on body composition, blood pressure and metabolic parameters. These effects can reduce cardiovascular risks later in life.

On Body Composition

During rhGH treatment, an increment in caloric, fat, carbohydrate and protein intake was reported in SGA children compared to baseline, with a reduction in total body fat mass, increase in lean body mass (57-59) and a significant decrease in leptin level (57,60). The degree of these effects was associated with younger age at onset and longer duration of treatment, height gain (58), and higher rhGH doses (59,61,62). After rhGH cessation, total fat mass increases and lean body mass decreases (58,61,63), and similar total fat mass and fat distribution were observed between adults born SGA previously treated with rhGH, untreated adults born SGA without CUG and those with spontaneous CUG (61), suggesting that treatment had no unfavorable effects on body composition. However, when analyzing lean body mass, lower values were found for young adults born SGA without spontaneous CUG, both treated with rhGH and those without treatment, compared with adults born SGA who had spontaneous CUG and adults of normal height born AGA. As a group, adults born SGA had lower lean body mass than AGA born adults (61).

On Bone Mass

Bone mass and bone mineral content (BMC) are lower in SGA children. During treatment, rhGH improves BMC and bone mineral density (BMD) (64,65). Smeets *et al*. (64) showed that BMD remained stable until 6 months after rhGH cessation, followed by a decrease probably due to loss of pharmacological effects of rhGH. At 5 years after cessation (mean age 21 years), previously rhGH-treated adults had similar BMD as untreated short SGA adults, SGA adults who had spontaneous CUG and AGA controls (64).

On Cardiac Function, Blood Pressure and Carotid Intima Media Thickness

Aurensanz Clemente *et al*. (66) and Faienza *et al*. (67) reported subtle vascular and cardiac morphological and functional abnormalities in SGA subjects compared to those

born AGA, with a thicker intraventricular septum and impaired right ventricular systolic function. It was suggested that rhGH treatment could lead to an improvement in the right ventricular systolic function (66). Systolic (SBP) and diastolic (DBP) blood pressure decrease during rhGH therapy, but 6 months after treatment cessation, both SBP and DBP increased with further decrease in subsequent 18 months (68). At 5 and 6.5 years after rhGH interruption, SBP was lower and DBP was similar or lower to baseline (35,68) and markedly lower than those of untreated short adults born SGA (35). Carotid intima media thickness (cIMT), a sensitive marker of atherosclerosis risk, did not change significantly at any time after rhGH interruption, and it was similar between adults born SGA rhGH-treated, those with normal height and spontaneous CUG or AGA controls. However, untreated short adults born SGA had higher cIMT than other groups (67,68), and it was suggested that rhGH could be beneficial for cardiovascular conditions.

On Glucose Tolerance and Insulin Sensitivity

Infants born SGA might have insulin resistance since their first years of life and impaired beta-cell function at 4 yr of age have been described (69). Insulin resistance at 6 years of age was reported, mainly if catch-up in height was accompanied by an increase in body mass index (70). An increase in fasting insulin and glucose levels and insulin resistance has been reported during rhGH (71), with higher glycated hemoglobin (HbA1c) levels in those with higher rhGH doses (0.067 mg/kg/day), though in the normal range (72). These effects were reversible with treatment discontinuation and 5 to 6 years after stopping treatment, insulin sensitivity was not different compared with that in adults born SGA who remained untreated (26,35).

On Lipid Profile

Serum apolipoprotein A1 (ApoA1) and high-density lipoprotein cholesterol (HDL-c) levels increased during 12 months of rhGH, and low-density lipoprotein cholesterol (LDL-c), apolipoprotein B (ApoB) and ApoB/ApoA1 ratio decreased (73,74). The later ones are better predictors of cardiovascular diseases than cholesterol (73), and therefore, it is suggested that rhGH treatment might reduce cardiovascular risk. After 5 years of rhGH treatment, total cholesterol (TC) and LDL-c were lower than baseline, especially in SGA children who received the higher dose (0.067 mg/kg/day). Also, HDL-c were higher after 5 years of rhGH in the 0.033 mg/kg/day group, with no changes in the higher dose group (72). Triglyceride, TC and LDL-c levels increased until 18 months and remained stable from 2 to 5 years after the end of treatment with a positive association to fat mass. At a mean age of 21 years, adults born SGA treated with rhGH had lower TC and LDL-c and higher

Table 2: Being born SGA and metabolic consequences of rhGH therapy

	SGA vs. AGA subjects	Short-term rhGH effects	Long-term rhGH consequences (5-6 years after rhGH cessation)
Body composition	SGA: low lean body mass, high total fat mass, high central adiposity SGA-CUG: high % fat mass	Increase of lean body mass Reduction of total fat mass Reduction of cental fat tissue	Increase of total and % fat mass Reduction of lean body mass Lower lean body mass vs. SGA-CUG Similar fat distribution vs. SGA-CUG and AGA
Bone mass and density	Similar	Increase	Similar vs. short-SGA, SGA-CUG and AGA
Cardiac function	Morphological and function abnormalities	Improvement in right systolic function	Not described yet
Blood pressure	SGA: high SBP and DBP	Reduction of SBP and DBP	Lower SBP and DBP vs. short-SGA
Carotida intima media thickness (cIMT)	Short-SGA: higher	Reduction	Similar to SGA-CUG and AGA
Insulin sensivity	SGA: lower	Higher glucose levels and insulin resistance Higher HbA1c levels	Similar to SGA-CUG and short-SGA
Lipid profile	SGA: high or similar TC and LDL-c	Increase of apoA1 and HDL-c Reduction of apoB and LDL-c	Lower TC and LDL-c vs. baseline, SGA-CUG and AGA Higher or similar HDL-c vs. baseline and short-SGA

Abbreviations: SGA, Small for Gestational Age; rhGH, Recombinant Human Growth Hormone; AGA, Appropriate for Gestational Age; SGA-CUG, SGA with Spontaneous Catch-Up Growth; short-SGA, untreated Short Adults born SGA; SBP, Systolic Body Pressure; DBP, Diastolic Blood Pressure; HbA1c, Glycated Hemoglobin; TC, Total Cholesterol; LDL-c, Low Density Lipoprotein Cholesterol; HDL-c, High Density Lipoprotein Cholesterol; ApoA1, Apolipoprotein A1; ApoB, Apolipoprotein B.

HDL-c levels than SGA adults who had spontaneous CUG and similar lipid profiles to AGA adults. The untreated short adults born SGA had the highest TC and LDL-c levels when compared with the other groups (68), suggesting that CUG induced by rhGH was beneficial for lipid profile.

In conclusion, treatment with rhGH for short children born SGA is beneficial to increase adult height, mainly if initiated before puberty. Although the effects of treatment on body composition are not permanent, rhGH may have beneficial effects on bone mass, blood pressure and lipid profile, as well on cardiovascular risks in adults born SGA.

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Growth Hormone Treatment for Idiopathic Short Stature

Wayne S. Cutfield[1], BHB, MB, ChB, MD, Benjamin B. Albert[2], BHB, MB ChB, PhD

[1]Professor in Paediatric Endocrinology, University of Auckland, New Zealand, [2]Maurice Paykel Research fellow in Paediatric Endocrinology, Liggins Institute, University of Auckland, New Zealand

Corresponding author: Wayne S. Cutfield, BHB, MB ChB, MD, Liggins Institute, University of Auckland, Private Bag 92019, Auckland, New Zealand, Tel: +64-9-923 4476, E-mail: w.cutfield@auckland.ac.nz

Abstract

ISS is the commonest cause of short stature and poor growth and is arbitrarily defined as a height < -2 SDS without an identified cause. ISS consists largely of normal children with the remainder unrecognised conditions, mainly syndromes and genetic (monogenic and polygenic) causes. Growth response to rhGH is widely variable reflecting the heterogeneity of ISS. Further identification of genetic causes of ISS will better characterise treatment response.

rhGH during childhood has been shown in RCTs to improve adult height by approximately 4 cm which is less than seen in other treated growth disorders. Factors that influence response include; younger age, longer birth length, lower height compared to mid-parental height, delayed bone age and larger rhGH dose.

The evidence that short stature is associated with psychological well-being and quality of life is minimal and that rhGH could improve this is scant. Further research in this area is urgently required.

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Introduction

Growth is the most important indicator of the health of a child, with short stature one of the commonest presentations to paediatric endocrinologists. Whilst more than 90% of these children do not have an identified underlying disorder and are categorised as having idiopathic short stature (ISS), it is important to recognise that children with underlying significant illness are frequently short and grow slowly.

Definition

To establish consistency in the definition and management of children with ISS an international consensus meeting was held in 2008 (1). There was broad agreement that ISS is arbitrarily defined as a condition in which the height of an individual is more than 2 SD score (SDS) below the corresponding mean height for a given age, sex, and population group without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities. Specifically children with ISS have normal birth weight and are not growth hormone (GH) deficient (1). The peak stimulated levels of GH used to define GH deficiency are very arbitrary and in most countries is a plasma GH <10 mcg/l in response to two stimuli with or without sex steroid priming. Such liberal criteria to define GH deficiency inevitably means

that within the idiopathic GH deficient group there are many children who actually have ISS. Within ISS the largest subgroup (up to 80%) are children with familial short stature (FSS) and those with constitutional delay of growth and puberty (CDGP) (2). Those children with FSS have shorter parents and their height is within the mid-parental height percentile range. Conversely those with CDGP have a delayed bone age, delayed onset of puberty, have a greater difference between their current height and the mid-parental height and attain an adult height greater than expected based upon current height SDS. There has been debate as to whether children with FSS and CDGP should be excluded from ISS (3), however, there is blurring across these two groups and others within the ISS group. FSS and CDGP are defined by arbitrary criteria across a growth and pubertal age continuum. A substantial portion of short children have the combination of FSS and CDGP leading to more obvious short stature. Predictably adult height in those short children with FSS was closer to mid-parental height (-1.5 to 3.0 cm) than those with CDGP (-7.5 cm) (4).

Diagnosis

The diagnosis of ISS is one of exclusion of other subtle chronic diseases. A thorough history, examination and screening should at minimum include investigation for coeliac disease (antibodies) chronic inflammatory disorders and anaemia (complete blood count and erythrocyte sedimentation rate) (5), Turner Syndrome (karyotype), hypothyroidism (TSH and T4), chronic renal disorders (urinalysis and urine culture) and renal tubular acidosis (serum bicarbonate) (6). In many centres a pragmatic approach is taken to only perform these screening tests if a child displays a poor height velocity (<25th percentile for age) over ≥six months as chronic illness typically leads to short stature due to a sustained poor height velocity (5,7). This is highlighted by Sisley *et al* who identified new pathology from screening investigations in only 1.3% of children with short stature and normal height velocity referred to a regional paediatric endocrinology centre (8). Thus the cost was very high ($US105,107) for each new diagnosis identified in this group (8).

There are a number of disorders that are excluded from ISS and include, idiopathic GH deficiency, small for gestational age, dysmorphic syndromes and skeletal dysplasias. However, identification of these disorders is not always clear-cut and some affected children will have an incorrect presumptive diagnosis of ISS. Distinction between ISS and idiopathic GH deficiency is based upon arbitrary cut-off values for stimulated GH levels, which may yield high rates of false positive test results when not performed with sex steroid priming (9,10). Whilst SGA is arbitrarily defined based upon auxological criteria (11), there is actually a continuous relationship between lower birth weight and shorter stature. Similarly, with increasing short stature in parents there is greater likelihood of short stature in SGA children (12). Moreover, published charts for birth weights and lengths for gestational age vary considerable and many countries do not have country specific charts. Within the ISS group there are inevitably children with a subtle unrecognised syndrome and children with subtle skeletal dysplasias in which auxological evidence of skeletal disproportion may be minor or absent. All short children should have assessment of skeletal proportion most commonly performed by standing and sitting heights. When the phenotype is subtle hypochondroplasia is easily misdiagnosed as ISS (14). Furthermore, a novel mutation in the FGFR3 gene has been shown to cause proportionate short stature (13), thus hypochondroplasia should be considered in children who have a short parent or whose height is discordant to parents' heights, even if they do not have skeletal disproportion.

Genetics

Growth is a complex process regulated by a large number of genes that exert their influence in the prenatal and post-natal phases. It has been proposed that in ISS there are three broad genetic influences; polygenic, monogenic and specific genes associated with growth plate development (3). The vast majority of ISS patients have polygenic short stature and have inherited many common gene variants that each contribute a small effect size that in combination lead to short stature. The combined effect of these variants accounts for up to 10% of the variation in adult height. Other unidentified common gene variants are likely to contribute a further 40% to adult height variation (14). The remaining genetic variability in adult height is likely to include non-coding regions that influence the expression of genes regulating growth through their effects on the spatial confirmation of DNA (15).

Unlike polygenic short stature, those with monogenic short stature have a single unrecognised gene defect that has a large effect on growth. These gene defects may not be recognised because they are rare and lack an obvious phenotype or because the patient has a milder form of the typical phenotype (16). The latter was illustrated by large scale next generation sequencing in 192 children with ISS in whom 3 had mutations in the PTPN11 gene which is associated with Noonan Syndrome (17). The phenotypic features of Noonan Syndrome vary widely and as a consequence it is difficult to know what proportion of ISS children may have a milder form of Noonan Syndrome (18). With greater use of genetic testing extending to whole exome sequencing in ISS more children will have their diagnosis changed to a monogenic growth disorder.

One of the more common monogenic causes of ISS is heterozygous mutations in the short stature homeobox-containing (SHOX) gene. This gene is located on the short arm

of both the X and Y chromosomes and escapes X inactivation. Whilst the penetrance of SHOX mutations are high, clinical expression is variable becoming more pronounced with age with increasing progressive mesomelic disproportion and being more severe in females (19). Growth failure begins early in childhood with an eventual mean adult height of -2.2 SDS (20). Screening of ISS cohorts has revealed a wide variation in the frequency of SHOX mutations affecting between 2 to 15% (21-23). The increased prevalence in more recent studies reflects the increasing sophistication of genetic testing and recognition of the functional significance of the enhancer region downstream of the SHOX gene (19). In younger children (< six years of age) with SHOX mutations there is short stature but typically absence of skeletal disproportion which develops in later childhood, thus diagnosis can be difficult in a childhood with short stature and normal examination and history. Several investigator groups have established prediction equations to identify those short children who have SHOX mutations (20-23). These include the sitting to standing height ratio, arm span to height ratio and a complex formula including multiple auxological variables (20-23). Whilst negative predictive value has been reported as very high (correctly identfying those without SHOX mutations), positive predictive values (correctly identifying those with SHOX mutations) for these formulae are low because younger children have little or no skeletal disproportion (20-23).

In recent years C-naturietic peptide (CNP) has been shown to be a regulator of the growth plate (24). Heterozygous mutations of NPR2 (the receptor for CNP) appear to explain up to three percent of ISS and possibly more if a similar phenotype is seen in a parent (25). These heterozygous mutations lead to a similar phenotype to heterozygous SHOX mutations with subtle disproportionate short stature principally due to mesomelic shortening (26).

Hypochondroplasia shares many of the skeletal characteristics of those with SHOX mutations, notably mesomelic shortening. It is due to defects in the gene FGFR3 which are present in up to 70% of those with a phenotype consistent with hypochondroplasia (27). A novel FGFR3 mutation associated with proportionate short stature was recently described, thus it is likely that hypochondroplasia is underdiagnosed and characterised as ISS (13).

Sex chromosomal abnormalities causing short stature without other phenotypic changes are not uncommon and include Turner Syndrome and males with a 45XO/46XY karyotype. In a large cohort of short children referred to a genetics clinic 7.7% of females had Turner Syndrome and 2.8% of males had karyotype abnormalities including 45XO/46XY (28).

Genes that influence growth could have their effects through regulating the rate of skeletal maturation and the timing of puberty. To date understanding genetic and epigenetic regulation of the timing of growth has yielded limited information. Single nucleotide polymorphisms (SNPs) have been identified that link adult height with the timing of peak infancy and pubertal height velocity (29). Whilst imprinted genes have been shown to be associated with adult height, early environmental epigenetic changes are relatively unexplored (30).

With the rapid evolution of more affordable genetic testing from genotyping arrays through to whole exome sequencing, it is now possible to consider genetic testing in children with ISS. Which of these children should undergo genetic testing? Genetic testing of ISS children will identify uncommon monogenic defects with a large effect. Conversely, polygenic short stature is difficult to identify and of limited clinical utility. The most obvious indications for genetic testing are extreme short stature (height SDS <-3), skeletal disproportion and those who have heights very discordant (>2 SDS lower) to one or both parents (16). Those children with extreme short stature with similarly short parents are more likely to have polygenic short stature and genetic testing of these children is of little benefit. Dauber has developed a diagnostic algorithm for the genetic diagnosis of short stature across all short stature disorders to guide clinicians (16). There are a number of benefits in establishing a genetic diagnosis of monogenic short stature in a child with ISS. These include highlighting comorbidities and future health risks, an end to the diagnostic journey of uncertainty for the parents and child, and the opportunity for genetic counselling.

Growth Hormone Treatment

The earliest report by Raben of GH treatment in children with ISS demonstrated a doubling in short term height velocity in five children with thrice weekly cadaveric GH (31). Interestingly, the response was similar to that seen in “pituitary dwarfs” and suggested benefit to children with growth disorders (31). Twenty years later another small cohort of short non-growth hormone deficient children (ISS) were treated with cadaveric GH for six months at 0.1U/kg thrice weekly. Of the 14 children treated only six had an increase in height velocity (2.2 to 4.2 cm/yr) which was comparable to 14 GH deficient children (32). Thus it appeared that ISS children had a variable response to GH therapy which included responders and non-responders.

In view of the limited supply of cadaveric GH, clinical studies of GH treatment in ISS were severely constrained until the successful production of recombinant human GH (rhGH) in the late 1970s (33,34). From 1985 rhGH has been produced in large quantities and with almost unlimited availability there was rapid expansion of clinical trials of rhGH for growth disorder including ISS.

Early rhGH studies looked at short term efficacy (6-12 months), factors that were associated with better growth response and safety. These studies influenced the decision by the US FDA to approve rhGH treatment for children with ISS who had marked short stature (height SDS <-2.25 below the mean for sex and age), with a growth rate not expected to lead to an adult height in the normal range, and in whom other causes of short stature were excluded. Subsequently several other countries have approved rhGH treatment for children with ISS with a range of entry criteria; for example height below the first percentile in Australia, and height SDS <-3 with height velocity <25th percentile in New Zealand. Doses of rhGH approved for use in these countries vary markedly from approximately 32 mcg/kg/day to 67 mcg/kg/day.

Whilst short term studies show acceleration in growth they do not indicate whether treatment throughout childhood leads to greater adult height. In a recently updated systematic review of rhGH treatment to adult height in ISS there were 19 long-term trials identified, 10 of which were controlled, with just three randomised controlled trials (RCT) (35-37) and seven non-randomised trials (non-RCTs) (38). Unfortunately, most of these studies were of low quality (39) with no single RCT of high quality. Overall the effect of rhGH on adult height in ISS was less than for other currently licensed growth disorders (40). From the seven non RCTs the overall effect of rhGH to adult height was +0.45 SDS (approximately 3 cm) and from the three RCTs +0.65 SDS (approximately four cm with 95% confidence intervals 0.4 to 0.91 SDS) (38). A wide range of rhGH doses and dosing regimens as well as age at recruitment have been used in ISS studies, more so than for most other growth disorders which makes assessment of response complex. For example in the three RCTs doses used varied from 33 to 74 mcg/kg/dose administered from three times weekly to daily (35-37).

Combined, these three RCTs include only 115 children of which half were treated with rGH. Response to rhGH treatment was very wide both within and across studies and was more variable than that seen in other growth disorders. This may in part reflect the inclusion of children with bone age delay who are heavily represented in the ISS study groups.

In the largest RCT, performed by Albertsson-Wikland *et al*, there was a greater response in the high rhGH dose (67 mcg/kg/day) compared to the low dose (33 mcg/kg/day) group (36). In both non-familial short stature and familial short stature subgroups the higher dose led to a +0.4 to +0.5 SDS increase in adult height compared to the lower dose. These groups were treated with rhGH throughout the treatment period of five to six years (36).

In a further small long-term RCT Van Gool *et al* did not find an increase in adult height with rhGH treatment. However, the treatment strategy which included pulses of treatment in the first year and treatment solely during the prepubertal phase is an experimental rather than conventional approach to treatment (41). Treatment was high dose (75 mcg/kg/day at initiation and 64 mcg/kg/day by treatment end) and was found to accelerate bone age but was not associated with an increase in adult height (41).There were three month pulses on and off rhGH in the first year which is usually the year associated with the greatest treatment response, potentially diminishing the treatment effect.

There is inequity in access of GH treatment in ISS. The reasons for this inequity are complex and include; social and financial disadvantage, family and community attitudes and expectations, and fear of drug treatment. Those children with ISS referred for short stature and treated with GH are more likely to be boys, come from more affluent families and in Western countries are more likely to be Caucasian. In the US in a large primary care population girls were equally likely to have short stature as boys yet 74% of those with ISS treated with GH were boys (42). Furthermore, those treated with GH were two to three times more likely to be Caucasian and twice as likely to have private health insurance (42).

Interestingly, approval in the US for GH treatment for children with ISS did not appear to increase the referral rate for children with short stature in at least one regional centre (43).

Growth Hormone Treatment Strategies

Several quite different rhGH treatment strategies have been used in ISS which include weight or surface area based dosing and individualised treatment based upon either prediction modelling or IGF-I titration of rhGH dosing (44-47). Virtually all treated ISS children receive standard dosing based upon weight or surface area as has been performed in clinical studies to date and has been approved by regulatory bodies. Individualised treatment has not been widely adopted despite the potential advantages for maximising growth promotion and cost-effectiveness.

Ranke and Lindberg have developed growth response prediction models for a range of growth disorders from the KIGS international growth database. This is the world's largest GH treatment database in children and creates statistical power from the large number of treated participants, but is somewhat limited by the lack of standardisation across centres for laboratory and radiological variables and the restricted range of variables available for the models. Nevertheless these models have the benefits of an optimised as well as a more realistic expectation of initial growth response.

Initially Ranke and Lindberg developed a first year growth response prediction model for ISS children based upon data from 657 prepubertal children treated with a relatively low dose of rhGH (27 mcg/kg/wk in 6 doses) (48). Predicted first

year height velocity in cm per year = 9.3 + (-0.3 x age at start in years) + (0.31 x weight SDS at start) + (0.74 x rhGH dose in mg/kg/wk) + (-0.33 x height SDS – mid-parental height SDS at start of rhGH) ±1.2(48).

This four parameter model could only explain 39% of the variability in response to rhGH. Most of this variability was due to younger age at the start of treatment (21%) with larger rhGH dose (11%), heavier body weight (4%) and greater discordance to parents heights (as a SDS, 4%) also contributing to variability in response. In addition a regression equation was developed for adult height SDS.

Adult height SDS = 1.26 + (0.37 x mid-parental height SDS) + (-0.05 x age at start in years) + (0.70 x height SDS at start) + (0.24 x student residual for first rhGH treatment year) (45,48). The error in height was 0.63 SDS; with the equation explaining 64% of variability. Thus those that achieved the greatest adult height had taller parents, were younger and/or taller at the start of treatment and had a larger initial growth response in the first year of rhGH treatment (45,48). These two equations for initial response and adult height underline the importance of the first year of rhGH response in the overall benefit in treatment to adult height.

Cohen has promoted the concept of using rhGH to titrate IGF-1 to a target level for each individual in an attempt to maximise the growth promoting effects of circulating IGF-I. They demonstrated that in 102 ISS children the rhGH dose could be titrated to achieve a target IGF-I level (either 0 or +2 SDS) over one year (47). Those that reached the higher IGF-I level (+2 SDS versus 0 SDS) received a much larger rhGH dose (114 versus 32 mcg/kg/day) and had a greater increase in height SDS (+1.47 versus +1.04 SDS) (47). The latter illustrates that the relationship between rhGH dose and response is not linear and needs to be considered in the overall cost of therapy. The study assumes that all patients are compliant with treatment, however it is clear that non-adherence with rhGH treatment is one of the main causes of variability in growth response (49). Interestingly, the data showed that ISS children do indeed have partial resistance to rhGH treatment. In all three groups (conventional treatment, low IGF-I and high IGF-I targets) GH deficient children had an approximately 30% greater increase in height gained compared to the ISS group (47).

Other Growth Promoting Strategies

A strategy to try to improve adult height in children with ISS who are receiving growth hormone is to aim to slow progression of bone age, delaying closure of the epiphyses, so that growth occurs over a longer period, albeit at a slower rate. Both aromatase inhibitors which inhibit estrogen production (in boys), and gonadotrophin releasing hormone (GnRH) agonists, which halt puberty have been used.

Aromatase inhibitors used as monotherapies in boys with ISS (50,51), or in combination with GH in those with GH deficiency (52) have been shown to improve predicted adult height by approximately 5cm (50-52). However, the effects on adult height are unknown. This difference is important as height prediction is insufficiently precise and may be unreliable in the context of treatments that interfere with skeletal maturation. In a recent study of pubertal boys with ISS the combination of rhGH and an aromatase inhibitor lead to greater absolute height change but only a trend to greater near adult height than rhGH alone (53). The overall difference in height gained between the rhGH and rhGH/aromatase inhibitor groups was small (1.9cm) (53), so that this may not have persisted if the children were followed to their actual final adult height. There has been concern raised about the safety of aromatase inhibitors since a small study showed that 45% of letrozole treated children with ISS had mild vertebral deformities detectable by magnetic resonance imaging (54). The significance of these deformities is unknown, and multiple studies have shown that aromatase inhibitors used in short children do not reduce bone mineral density (50,51,53). Currently, it appears that the safety of aromatase inhibitors are not sufficiently established. They may lead to a small improvement in height, however adult height data is needed to confirm this.

There is limited evidence for the use of GnRH agonists in combination with growth hormone to improve adult height. Despite this, the combination has been used relatively often (55). Uncontrolled data from KIGS and NCGS databases would suggest poorer outcomes for these children, but are biased because only children with poorer prognosis are given the combination treatment (55). In a very small non-randomised study a GnRH agonist with rhGH led to greater gain over predicted height than rGH alone (56). However, a retrospective analysis showed children treated with the combination showed no difference than with rhGH alone (57). A recent randomised controlled trial was conducted in France, but concerningly, was prematurely stopped limiting analysis (58). Near adult height was very similar between groups, although this was determined for only a third of participants. There remains a need for a high quality adequately powered randomised controlled trial to determine the actual effects of adding a GnRH agonist to rhGH treatment in pubertal children with idiopathic short stature.

Factors Associated with Growth Response to Growth Hormone

Within the ISS group there is a very wide range in the short and long term response to rhGH treatment, more than that seen in other growth disorders. There are three broad areas that determine response to rhGH in ISS; polygenic variation in

growth responsive genes, pre-treatment clinical characteristics and compliance with rhGH treatment. The first of these categories was discussed in detail earlier.

As earlier outlined there are many studies that have sought to identify common gene variants influencing childhood height, however common gene variants could also explain response to rhGH. The concept of growth responsive genes to rhGH treatment (pharmacogenomics) in ISS was originally explored by Bougneres and colleagues (59). In a cohort of short children with a poor height velocity three groups were identified; GHR_{fl}/ GHR_{fl}, GHR_{fl}/GHR_{d3} and GHR_{d3}/GHR_{d3} (59). The cohort included 96 prepubertal ISS children treated for two years with rhGH. There are two common GH receptor (GHR) isoforms; the full length (GHR_{fl}) and exon d3 deletion (GHR_{d3}) isoforms, with both forms biologically active (60). Children who were homo- or heterozygous for GHR_{d3} had a growth response that was 1.7 to 2.0 times greater than those who were GHR_{fl} homozygous (59). However, two further short term studies have revealed inconsistent effects of GHR_{d3} on growth velocity following rhGH therapy in ISS children (61,62). Carrascosa showed no effect of the GHR_{d3} on response to two years of rhGH in 106 ISS children (61). Conversely in a small Korean cohort (52 ISS children), GHR_{d3} was associated with a slightly greater growth velocity after one year of rhGH treatment (62). GHR_{d3} is unlikely to explain normal variations in growth as the effect of the polymorphism on GHR sensitivity would be offset by alterations in GH secretion (63). Collectively these studies suggest a small influence of GHR_{d3} on initial growth responsiveness with rhGH treatment in ISS. It remains unclear what the magnitude of effect of this polymorphism is to rhGH treatment to adult height. Given that there are many polymorphisms regulating growth, it is difficult to untangle the effect of a single polymorphism when there are many at play that both positively and negatively regulate growth.

In assessing growth response to rhGH several groups have evaluated factors associated with greater response to rhGH. Identifying such factors may help guide clinicians and parents in predicting the likelihood of response to rhGH treatment. The Swedish Study Group treated an ISS cohort to adult height (64). They studied 108 prepubertal children randomised to three groups; control untreated, rhGH 33 mcg/kg/day and rhGH 66 mcg/kg/day to adult height. Sixty-two percent of the variance in height gain (expressed as change in height SDS), could be accounted for by four factors; a larger increase in IGF-I SDS from baseline to adult height (28% of variance), greater bone age delay, longer birth length and higher rhGH dose (64). Baseline IGF-I alone was not associated with growth response, nor was short term increase in IGF-I at one and three months (64). This indicates that initial change in IGF-I cannot guide which children will achieve long term growth response to rhGH. From the KIGS study group there 425 ISS children treated with rhGH to adult height (48). The major predictors of adult height were; early age at the start of treatment, higher doses of rhGH used, longer birth length, lower height compared to mid-parental height and delayed bone age (48).

One of the major factors affecting response to rhGH is treatment non-adherence (49). In a national prospective study of adherence based upon vials returned monthly for six months, 66% of subjects missed >1 dose/week irrespective of the underlying diagnosis (49). Treated subjects included those with ISS. Importantly, there were marked reductions in height velocity over the monitoring period between those with high compared to medium or low compliance (49). In GH deficiency non-adherence was more common in pubertal rather than prepubertal children (65,66). A review of rhGH adherence identified a large number of associated factors that were inconsistently identified (67). Factors that were identified in some studies that might help inform strategies to improve adherence included; less affluent families, poorer understanding about treatment, inadequate training to use the rhGH delivery device, difficulty with injections and misunderstanding of the consequences of missed doses (67). Anecdotally clinicians are often unaware of children non-adherent to rhGH treatment.

Psychological Outcomes and Wellbeing

As ISS is not a disease, the major justification for rhGH hormone treatment has been to increase childhood and adult height which is presumed to improve well-being and psychosocial outcomes. However, the evidence that short stature is associated with social, psychological and physical well-being, and that rhGH treatment is beneficial for treating short children is limited (68).

Short stature is associated with poorer IQ and educational achievement in childhood, but the effect is small and may not be causative. Two population-based studies from the United States (69) and the United Kingdom (70) show that short stature is associated with poorer IQ and educational achievement. However, the effects of socioeconomic factors are much stronger (70), and it is possible that the relationship could be explained by other confounding factors such as aspects of early life health and nutrition (68). Further, while those with short stature may have reduced academic achievement compared to normal height controls, most studies have shown that in children with short stature IQ is at or above population means (71).

Short stature in childhood is associated with juvenilization and a small increase in the risk of teasing, but positive peer relationships do not appear to be affected. More than half of children referred for assessment of short stature were regularly teased about their height (68). However, in a population-based study, short children had only marginally greater levels

of peer victimisation than other children (72). This reflects that bullying is common, and suggests that for short children height often becomes the subject of bullying but is not the sole cause. In school-based studies height was not related to popularity (72,73), total number of friends or whether friendships were reciprocated, and being short did not affect reputation, social acceptance (73), or scores in the relationship with peers questionnaire (72). Together this suggests that the social impacts of short stature in childhood are small.

Many studies have reported that short stature is associated with behavioural and psychological problems, however, this is likely to have been overestimated. Short stature has been reported to be associated with high incidences of behavioural disorders (74,75), but studies that have focused on children with underlying abnormalities (such as hypopituitarism, chromosomal disorders and skeletal dysplasias), may not reflect children with idiopathic short stature who are otherwise normal (76). Previous studies may also have been biased by relying on parental reports. When parental reports are used short stature has been associated with behavioural and psychological problems (77-79), but parental reports have been demonstrated to overestimate behavioural problems in children with short stature (77). In addition, studies based in clinic patients may overestimate the psychosocial problems of short children in the community. This was demonstrated by a study that compared children with short stature who were referred for assessment, with those not referred, and to those of normal stature (80). While, those not referred were no different from a control group, the referred group had worse psychosocial functioning. This suggests that psychosocial problems may increase the chance of referral, but are not themselves caused by short stature (80). Population based studies are less prone to these biases, and suggest that short stature is not associated with adverse behavioural of psychological outcomes (72).

A similar pattern emerges when quality of life is assessed. Population based studies showed no association between height and depression (72) or health related quality of life(HRQoL) (81). In some European studies based in clinic patients with short stature, the shortest children had worse quality of life measures (79,82), but this was not found in other studies (83,84). In surveys of children in Swedish clinics, short children had normal well-being, but their parents rated it lower (85,86). Thus while it may be that there is a small reduction in HRQoL in the shortest children, the effect is small, is overestimated by parents and for most short children HRQoL is within normal limits.

In adults, population-based studies have shown conflicting estimates of the relationship between short stature and HRQoL. A UK based study showed reduced quality of life in a number of physical domains with mild short stature (SDS <-2)(87) with no effect on indices related to anxiety or depression. However, this study did not control for differences in socioeconomic or health status which may have led to overestimating the negative effect of short stature (88). A French study controlling for such factors found height to be a very poor predictor of HRQoL, with lowered physical functioning occurring only with extreme short stature (Height SDS <-3.8 for men and <4.2 for women) (88). Only a very small proportion of children with ISS will achieve an adult height this low, suggesting that for the vast majority, HRQoL will be unaffected in adulthood. A large American poll looked at the relationship between height and a simple self-rating of how good life was. While taller adults rated their lives as marginally better, this effect became trivial once socioeconomic status, education and income were accounted for (89).

Many other benefits have been associated with greater height including greater income and education (90), being more likely to have a prestigious job (91), and reduced risk of suicide (92). However, in all such associations it is difficult to assign cause to height itself, as confounding factors such as childhood health, socioeconomic status, and intelligence may be more important. Thus it is not at all clear that improving height in children with short stature would be expected to improve their happiness, quality of life or success as adults.

Given relatively poor evidence that ISS is associated with psychosocial problems and reduced quality of life, there may be little scope for growth promoting therapies to improve this domain. In fact, there is little specific evidence that rhGH treatment improves quality of life or psychosocial problems. The vast majority of reports come from studies with substantial risk of bias, as they are predominantly non-randomised and not blinded (93). One double-blind placebo controlled study has been conducted that was designed to assess psychological and growth outcomes (94). However, participation in the psychosocial assessment was poor, and the study was stopped early. Treatment with rhGH was associated with a trend to reduction in problem behaviours as reported by the parents (94). As it is unlikely that further randomised placebo controlled studies like this will be conducted (93), the state of evidence is unlikely to dramatically improve.

When considering the potential for rhGH to improve psychosocial outcomes one must also consider that within the diagnosis of ISS, many will have underlying genetic conditions that could affect neurocognitive development and subsequently psychosocial outcomes and quality of life. It would be unlikely that this would be improved by rhGH.

Conclusions

ISS is an amorphous group composed of mainly short normal children (familial short stature and CDGD), those with unrecognised syndromes and monogenic and polygenic causes of short stature. The latter is a major contributing cause

to short normal children. Through whole exome sequencing more genetic causes of short stature will be elucidated and the growth patterns and responsiveness to rhGH treatment better understood. rhGH has a modest long term height benefit for ISS children that approximates four cm and is less than other disorders currently treated with rhGH. The addition of aromatase inhibitors or GnRH analogues to rGH in pubertal children may have a small additive effect on growth potential, but adult height data is limited. There are pre-treatment characteristics of ISS children associated with greater long term growth response that will guide expectations about treatment and may influence treatment decisions. Current studies of the psychological and HRQoL effects of short stature applied to ISS children suggests they are likely to be minimally affected and that treatment with rhGH makes scant difference. However, the data in this area is very limited in these children prior to and more so following rhGH. More rigorously conducted studies are urgently required to assess the true value of rhGH treatment in these children beyond cm gained.

Disclosure

Wayne Cutfield is a member of the KIGS Science Strategy Committee and Benjamin Albert has no conflicts to declare.

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Growth Hormone Treatment for Achondroplasia

Tohru Yorifuji, MD, PhD, Shinji Higuchi, MD, Rie Kawakita, MD

Division of Pediatric Endocrinology and Metabolism, Children's Medical Center, Osaka City General Hospital, Miyakojima, Osaka, Japan

Corresponding author: Tohru Yorifuji, MD, PhD, Division of Pediatric Endocrinology and Metabolism, Children's Medical Center, Osaka City General Hospital, 2-13-22 Miyakojima-hondori, Miyakojima, Osaka 534-0021, Japan, Tel: +81-6-6929-1221, Fax: +81-6-6929-1090, E-mail: t-yorifuji@med.osakacity-hp.or.jp

Abstract

Achondroplasia (ACH) is the most common form of skeletal dysplasia causing rhizomelic, short-limb short stature. Short- and long-term clinical trials have been conducted with rhGH, with similar results across these studies. At supraphysiological dose of GH, height gain of 1-1.5 SDS on the population curve was observed during the first 1-3 years, which was then followed by a smaller increase in growth rate persisting for 5-6 years. These studies led to the approval of rhGH for ACH in Japan where rhGH has been used for 20 years at 0.05 mg/kg/day. Although the available data are still limited, compared to untreated controls, total gain in adult height has been greater in males than in females, reported at 3.5-8.0 cm and 2.8-4.2 cm, respectively. Serious adverse events have been rare although some were potentially life-threatening and need careful monitoring. These results should serve as a comparator for novel emerging treatments for ACH.

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Introduction

Achondroplasia (ACH) is the most common form of skeletal dysplasia with an incidence of 0.36-0.6 in 10,000 live births (1), and is characterized by short stature, rhizomelic limb shortening, genu varum, trident fingers, excessive lumbar lordosis, and relative macrocephaly sometimes in association with hydrocephalus (2-5). ACH is caused by a gain-of-function mutation in the fibroblast growth factor receptor 3 (FGFR3) gene which normally functions as a brake to the proliferation of chondrocytes in growth plates (6-10). Almost all patients have the p.G380R mutation (mostly c.1138G>A, a few with c.1138G>C) in the FGFR3 gene (2-5).

Affected patients present with linear growth failure starting in infancy and, in association with the blunted pubertal growth spurt, the final adult height is quite short at approximately -6 to -7 standard deviations (SD) below the average of normal controls (11-13); which, in Japanese people, corresponds to 130.2 cm (-7.0 SD) in males and 124.0 cm (-6.43 SD) in females (14). Importantly, in addition to the short-limb short stature, these patients could have other skeletal morbidities such as cervical cord compression caused by the small foramen magnum, or lumbar cord compression by the narrowing of the lumbar spinal canal (2-5,15,16,**figure 1**). The cord compression could lead to neurological symptoms such as numbness, paralysis, sleep apnea, or even sudden death.

Several methods exist to treat the short stature of ACH patients. Surgical treatment by limb-lengthening results in a considerable increase (10-20 cm) in height although the procedure is physically demanding to the patients and is not always free of complications (17-23). Medical treatment has centered around GH. At present, however, the use of rhGH for ACH has been approved only in Japan, and its use is not generally accepted as a standard treatment for ACH because of the lack of data showing a significant increase in adult height (24).

In Japan, rhGH was approved for use to treat ACH in 1997. In this paper, we review the literature on rhGH therapy for ACH, and then summarize the experience with rhGH therapy accumulated over the past 20 years in Japan.

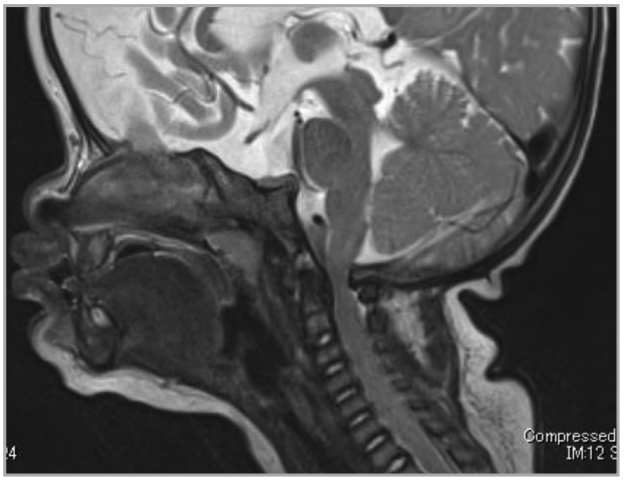

Figure 1. Spinal cord compression at the level of foramen magnum
An 8-months old patient with achondroplasia who required surgical decompression

Short-Term Treatment Outcome

The first attempt to treat ACH by GH dates back to 1933 when a treatment by pituitary extracts was first reported in a patient with presumably ACH (25). Since 1985, when rhGH has become available, trials to treat ACH patients have been conducted in many centers (26-39). The reported therapeutic outcomes until mid-2000's have been nicely summarized by Miccoli *et al* (40).

Although the dosage of rhGH and the ages of treated patients varied between studies, short-term trials of 1-3 year treatment have generally resulted in similar conclusions (40). When supraphysiological dose of rhGH, commonly used for other indications such as Turner syndrome, were used for prepubertal ACH patients, significant increases in growth rate were observed in the first year, typically from 4-4.5 cm/year before treatment to 6-7.5 cm/year, or an increase of 2-3.5 cm/year above the baseline growth rate. In the second year, the increase was less remarkable, typically 0.5-1.5 cm/year above the baseline growth rate (35,37).

Tanaka *et al*. treated 42 (16 males and 26 females) patients with ACH by rhGH at physiological (0.5 U/kg/week or 0.025 mg/kg/day, 8 patients) or supraphysiological (1.0 U/kg/week or 0.05 mg/kg/day, 34 patients) dosages for two years. In the latter group of patients, mean growth rate increased from 4.1+/-1.0 cm/year to 7.4 +/- 1.5 cm/year in the first year, and to 4.5+/-1.3 cm/year in the second year without major adverse events including the worsening of body disproportion (35). Although adult height and long-term consequences were not known at that time, the results of this study led to the approval of GH treatment for ACH in Japan in 1997 at a dose of 0.05 mg/kg/day.

Long-Term Treatment Outcome

There have been several reports showing the efficacy of long-term rhGH treatment of 4 to 6 years (36,37,39), again with similar results. When supraphysiological dosages of GH were used, the annual growth rate, which decreased in the second year of treatment to slightly above the pretreatment level, was maintained for up to 4-6 years of treatment. As a result, the height SD score as compared with normal children kept increasing until 3 years of treatment (typically +1.0-1.3 SD above baseline), then leveled off but maintained until 5-6 years of treatment. Since many of these patients were started on rhGH at the height of 5 to 5.5 SD below average, the height SDS at 4-6 years of treatment was maintained at 4 to 4.5 SD below average.

These results were further confirmed by the real world data accumulated in the ACH database of the Foundation for Growth Science in Japan. The Foundation for Growth Science is a public-interest incorporated foundation established in 1977 with the aim to collect pituitary glands inside Japan because of the limited supply of hGH, and then after the availability of rhGH, has been operating to promote proper use and research of rhGH treatment in Japan. The foundation has set up registries of patients who receive GH treatment in Japan for different indications including ACH. The ACH registry was established in 1997 when rhGH treatment was approved, and, as of May 2017, a total of 820 patients have been registered. **Figure 2** shows the growth outcome of male ACH patients who were treated by 0.05 mg/kg/day of rhGH for >5 years until near adult height. As described in previous studies, most of the patients experience rapid increase in achondroplasia-specific height SDS (ACH HtSDS) during the initial 1-3 years of treatment. Then, importantly, the increase was maintained beyond 5-6 years until near adult height. As described below, this translates into the increase in adult height in these patients.

Adult Height

Only a few data are available on adult height, partly because GH treatment has not been regarded as a standard therapy for ACH and has not been approved other than in Japan (24). So far, the only English publication on the adult height of rhGH treated children with ACH is from Japan by Harada et al (41). They reported height outcome of 22 patients treated with rhGH at 0.05 mg/kg/day for 10.7 +/- 4.0 years in males and 9.3 +/-2.5 years in females. They were treated until 16.2 +/- 1.3 years in males and 14.7+/-1.8 years in females, thus close to adult height. Since most (15/22) of them also received limb lengthening (tibial lengthening in 9 and combined tibial + femoral lengthening in 6), the difference between pre- and

Figure 2. Changes in achondroplasia-specific height SDS (ACH HtSDS) in male ACH patients treated for a long time (>5 years) until near adult height

Lines represent the changes of ACH HtSDS in each patient, and start at the ages of GH initiation. The ACH HtSDS was calculated using the gender- and age-specific growth chart for Japanese ACH patients (14,58).

post-surgical height were subtracted from the measured adult height. Then, by comparing the ACH HtSDS of each patient at the initiation and the end of rhGH treatment, they concluded that the actual adult height gain by rhGH was 3.5 cm in males and 2.8 cm in females.

Using the aforementioned data of the Foundation of Growth Science, we also analyzed the height of 39 patients with ACH (16 males and 23 females) who were treated by rhGH at 0.05 mg/kg/day until annual height gain of <2 cm, this time without limb-lengthening (42). Median duration of rhGH treatment was 8.05 years for males (95% confidence interval: 6.8-10.6 years) and 8.90 years for females (95% confidence interval: 6.6-9.0 years). In these patients, the median gain of adult height was 7.98 cm (95% confidence intervals: 6.58-10.63 cm) in males and 4.21 cm (95% confidence intervals: 2.51-7.81 cm) in females. These results translate into the adult height of 138.2 cm in males, and 128.2 cm in females, both corresponding to -5.6 SDS compared with normal controls.

The height gain observed in our study was significantly greater than that reported by Harada *et al* (41). The reason for this difference is unclear. Since patients with poor growth might have withdrawn from rhGH treatment or switched to surgical treatment before reaching adult height, poor responders might have been unintentionally excluded from the study. Actual average adult height gain, therefore, could be less than that observed in our study. However, it also has been reported that the limb-lengthening procedure itself reduces the growth potential of the treated bones (43,44). On 35 patients who underwent limb-lengthening and were observed until near adult height, Song *et al*. reported the total loss of height potential as 2.24 cm by tibial lengthening and 3.89 cm by combined tibial + femoral lengthening (44). Therefore, the results of Harada *et al*. might be underestimating the potential height gain by rhGH alone. Considering the loss of 2.24-3.89 cm to the observed gain of adult height, the differences between these two studies are smaller.

In both studies, male patients showed greater height gain as compared with female patients. The reason for this gender difference is currently unknown. Since most of the adult height gain by rhGH is made during the prepubertal treatment period, these differences might reflect earlier onset of puberty in females.

To summarize, rhGH treatment is effective to some extent for ACH, however, the attained adult height is still far below that of normal controls. When plotted against the growth curve of normal controls, the initial gain in HtSDS is gradually lost when the patients enter into puberty because of the absence of pubertal growth spurt in ACH.

Assuming a typical case of ACH, started on rhGH at 15 kg and treated for 10 years until 30 kg, even when we get the total

growth of 8 cm, the cost for growth would be 2,469,000 – 4,625,000 JPY/cm (approx. 19,000 – 35,500 USD/cm). Considering the fact that the adult height after treatment is still far below the normal range, the cost-benefit of rhGH therapy is a matter of debate.

Adverse Events

Another important aspect of GH therapy is its safety since patients with ACH can have a variety of co-morbidities as described above. In general, most of the reports showed that the supraphysiological dose of rhGH is well tolerated without major adverse events (28,31,33,35,37,39), However, Okabe *et al* reported an occurrence of sleep apnea and neurological deficits associated with atlantoaxial dislocation (27). In Japan, where rhGH has been approved for ACH for 20 years, the regulatory agency, the Pharmaceuticals and Medical Devices Agency (PMDA, corresponds to FDA in the US or EMA in EU) has accumulated a total of 660 adverse events associated with the use of rhGH as of April, 2018 (https://www.pmda.go.jp/safety/info-services/drugs/adr-info/suspected-adr/0003.html). Of these, 29 adverse events in 15 patients were related to its use for ACH, and only 5 patients discontinued rhGH treatment because of the adverse events: one for spinal canal narrowing, one for narrowing foramen magnum, one for kyphosis of the spine, and for the remaining two, the cause of withdrawal is unknown. Other common adverse events included hydrocephalus or tonsillar/adenoid hypertrophy. These, however, did not require discontinuation of rhGH. Overall, serious adverse events have been rare and the treatment has been well tolerated. The incidence of serious adverse events such as spinal cord compression might not be increased considering the number of patients treated in Japan. But still, the patients must be carefully monitored for the development of such events.

Since ACH is characterized by poor growth of long bones, changes in body proportion, theoretically, is expected by the rhGH therapy. With regard to body proportion, however, conflicting results have been reported, most of these reported unchanged body proportions (33-35,39) while some others reported worsening of the disproportion (36).

Future Perspectives

Recently, a number of novel treatment possibilities have been proposed for ACH (45), including C-type natriuric polypeptide (CNP) analogues (46-50), statin (51), meclozine (52-54), FGFR3-binding peptide (55), HDAC6 inhibitors (56), or tyrosine kinase inhibitors (57). Of these, a CNP analogue (BMN111, BioMarin), which inhibits the downstream signaling of FGFR3, already has been used in phase 2 clinical trials and is showing encouraging results. In the future, rhGH will be replaced by these novel therapies. However, the 20 years of experience of rhGH should serve as a comparator for these new modalities of therapy in terms of efficacy, safety, and economical advantages.

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Psychosocial Aspects of Short Stature and rhGH Treatment: Implicit Trends over 60+ Years

Melissa Gardner, MA, Teresa Scerbak, BS, David E. Sandberg, PhD

University of Michigan, Department of Pediatrics and Communicable Diseases, Division of Pediatric Psychology and the Child Health Evaluation and Research (CHEAR) Center, Ann Arbor, MI, USA

Corresponding author: David E. Sandberg, PhD, University of Michigan, 300 N. Ingalls, Rm 6C23, Ann Arbor, MI 48109, USA, Tel: 734-615-1958, Fax: 734-232-1400, E-mail: dsandber@med.umich.edu

Abstract

Between 1958 and today, advances in research and the clinical management of short stature with GH have occurred. Initially, limited supply of pituitary-derived hGH led to strict criteria for diagnosing GH deficiency and tightly controlled treatment protocols. With the advent of biosynthetic GH, the supply has increased, the number of indications for treatment has grown, and the focus of intervention changed from hormone replacement to treatment of short stature. Improved psychosocial adaptation is an underlying, albeit largely unspoken and inadequately researched, target of treatment. Complicating the ability to make a definitive statement on the effects of rhGH on psychosocial adaptation is the rigor of the psychological outcomes literature. A high risk of bias present in the majority of rhGH treatment studies on psychological outcomes substantially weakens confidence in their results. Studies that convincingly demonstrate, through rigorous research design and methodology, that the benefits of rhGH exceed the risks and burdens are needed.

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"Facts do not find their way into the world in which our beliefs reside; they did not produce our beliefs, they do not destroy them; they may inflict on them the most constant refutations without weakening them, and an avalanche of afflictions or ailments succeeding one another without interruption in a family will not make it doubt the goodness of its God or the talent of its doctor."

Marcel Proust, Swann's Way, 1908

Background

The beginnings of growth hormone (GH) treatment trace back to the late 1800's when the importance of the pituitary gland in growth was first recognized (1). Early research involving the pituitary and associated hormones led to unsuccessful attempts to treat growth failure with purified GH bovine pituitaries (1,2). Later research demonstrated the species-specific nature of GH. The first successful trial showing the growth-accelerating effects of human growth hormone (hGH) was published in a 1958 letter to the editor (1,3); results were replicated in several hGH trials published in 1960. Between 1963 and 1985, the US National Pituitary Agency (NPA) supervised almost all hGH treatment in the US and the costs of pituitary collection, hormone extraction, and distribution were borne by the National Institute of Health (NIH) (3). During this era,

supplies were limited; accordingly, treatment was restricted to patients considered to be GH-deficient according to restrictive criteria (4). The trade-off for receiving treatment at no-cost was that all patients were enrolled in NPA-reviewed research protocols for the first year of treatment. After the first year, patients could continue treatment until they reached 5 feet and research participation was no longer compulsory; however, supplies limited treatment to 8 out of 12 months of any given year. By 1983-1984, the supply of hGH had increased, allowing for all patients to be treated continuously up to a height of 5'6" in boys and 5'4" in girls (3). Parallels to the US experience existed in Canada, England and France where national programs sponsored treatment using strict guidelines to define GH deficiency (GHD), prescribe hGH treatment, and monitor growth (1,3,5). Guidance up through the mid-1980's focused heavily on physiologic measures (eg, growth velocity and safety-monitoring); with few exceptions, the potential psychosocial effects of hGH-accelerated growth did not enter as criteria for hGH treatment nor as outcome variables for research.

Several additional developments in science and industry exerted profound and evolving effects on the use of GH in treating growth failure. In brief, these included improvements in the purification of hGH (2), the first description of the direct expression of a DNA sequence coding for hGH in 1979 (6), the appearance of Creutzfeldt-Jakob disease that halted hGH distribution in 1985 (3), and the introduction of GH produced by recombinant DNA technology (i.e. recombinant human GH; rhGH) (1,4). Researchers had begun the process for establishing protocols for clinical trials of rhGH by 1981, pituitary-derived hGH distribution was halted in April 1985, rhGH clinical trials were completed and US Food and Drug Administration (FDA) approval was granted in October 1985 (2). Thus, with little delay, rhGH replaced hGH in treating GH-deficient youth. This replacement was not a 1:1 substitution; in fact, it marked a turning point in clinical management and research. In anticipation of FDA approval, a panel of experts in pediatric endocrinology, psychology, and bioethics and representatives from the National Institute of Health, FDA and pharmaceutical companies were convened in late 1983 by the Director of the National Institute of Child Health and Human Development to confer on uses and possible abuses of rhGH. Attendees predicted that the process for synthesizing rhGH would provide a virtually unlimited supply of GH which would become available outside the context of research protocols; they raised a number of diagnostic, therapeutic, and ethical questions concerning the rational and safe prescribing of rhGH (7).

Shortly before the 1985 sea change in the use of GH, the Lawson Wilkins Pediatric Endocrine Society (LWPES; name changed to the Pediatric Endocrine Society in 2010) and the AAP published a joint statement on the use of GH (8) that bears particular relevance to the issue of psychosocial aspects of hGH treatment. The 1983 statement included a careful review of the literature that touted 25 years of safety in treating children with GHD, but cautioned that use of hGH in non-GH deficient children should not be engaged in lightly as there were not enough data on the risk and benefits. The irony of marking 25 years of safety a brief two years before the first report of Creutzfeldt-Jakob disease in those who had been treated and withdrawal of hGH notwithstanding, the major issue to consider is that the literature review focused solely on physical growth and risks/benefits to physical health. Nowhere were any psychological, psychiatric, or social data presented; nevertheless, the 1983 report concluded with the following words:

As the investigational use of GH is expanded, researchers and clinicians should bear in mind that handicaps resulting from short stature often have psychological origins. Therefore, even for experimental studies, GH therapy should be restricted to children in whom it is judged that emotional status can be significantly improved. In selecting patients for GH trials the wise physician might adhere to the old adage, "If it ain't broke, don't fix it" (8) (pp. 894).

While the authors of the statement do not stand alone in such sentiments, it is, perhaps, the earliest and clearest written example that demonstrates a lack of precision in articulating the goals of treatment. Consider that all the research literature cited within the joint statement was focused solely on auxology and physical health; yet it concluded with a statement on psychological health. Further, consider the assumptions embedded within those concluding words. It speaks only of short stature. Growth failure / GHD is no longer the condition requiring treatment – instead it is short stature itself. Additionally, the conclusion is rationalized based on assumptions that those with short stature face handicaps, have problems of a psychological origin, and GH treatment improves emotional status. Where do these assumptions come from? And what can research tell us about their validity?

Sequelae of an Unlimited GH Supply on Clinical Management

rhGH was originally approved by the FDA to accelerate growth and increase adult height for those with GHD, but the unlimited supply made it possible to treat other pediatric conditions associated with short stature. short stature has been defined in the literature as height two or more standard deviations (SDs) below the mean for age- and gender-specific norms (4,9) (approximately the 2nd percentile). While short stature may reflect consequences of a wide range of pathologic states, including GHD, it may reflect healthy variation. FDA-approved indications for rhGH treatment have expanded to include: chronic renal insufficiency (1992) (10), Turner syndrome (TS;1996) (11), Prader-Willi

syndrome (PWS; 2000) (12), children born small for gestational age (SGA; 2001) (13), idiopathic short stature (ISS; 2003) (14,15), SHOX deficiency (2006) (16), and Noonan syndrome (2007) (17).

Unlike in GHD, in which hormone replacement served as a guiding principle of treatment, rhGH was never considered to be a treatment for the underlying cause of growth failure in these other conditions. Indeed, the words of the 1983 joint statement have become reality; clinical management has shifted to treating slow growth and "short stature" itself (18). Proponents of rhGH treatment in non-GHD children assert height, as an isolated physical characteristic, is associated with psychosocial morbidity and a justification for treatment. Others note controversy about such treatment (19) and question the evidence underpinning this quality of life rationale (20,21). At the same time, the threshold for a subnormal GH response to two provocative stimulation tests drifted upward – meaning that more children become eligible for treatment (4,22).

While the approved indications for rhGH treatment of short stature have changed, so too have the focus of guidelines. For example, the 1995 LWPES guidelines for the use of GH in children with short stature (22) began with a review of research on growth rate, final height, and adverse effects (e.g. decreased insulin sensitivity, pseudotumor cerebri, and slipped capital femoral epiphyses) in conditions where rhGH appeared effective at increasing adult height. It then shifted to reviewing data on growth velocity and height SD in children with conditions where rhGH has not yet been proven effective; ie, "non-GH-deficient short stature," constitutional growth delay, intrauterine growth retardation, glucocorticoid-induced growth failure, renal transplantation, Down syndrome, PWS, and Noonan syndrome.

After reporting on physiologic aspects of rhGH treatment, the report turned to data on psychosocial outcomes of short stature and rhGH treatment and summarized the data as such:

"Many psychologic studies… indicate that short stature per se does not result in negative psychosocial adaptation… However, those patients who are referred for evaluation of short stature may represent a group in which there are psychologic problems that have been attributed to the patient's size… It is important to address these issues early in evaluation to provide appropriate psychologic or behavioral intervention, rather than to assume that GH therapy will correct these difficulties" (22) (pp. 863).

Authors further noted that treatment with rhGH may have both positive and negative implications, enumerated several psychologic risks, then reviewed the scant extant research literature on effects of rhGH treatment on psychiatric symptoms, social adaptation, and vocational and educational achievement. It also was noted that, in contrast to studies in which positive short-term results were found regarding psychiatric and psychosocial adaptation, long term follow-up studies of individuals who had been treated with rhGH during childhood were less encouraging. Authors of the 1995 guidelines posited that rhGH treatment, on its own, may be insufficient in addressing psychological challenges observed in treated populations. Indeed, the Guidelines and Recommendations section stated, "research aimed at improving the diagnosis and treatment of growth disorders and at evaluating the impact of therapy on psychosocial function and quality of life is essential… Therapeutic trials should be ethically sound and should include appropriate control groups" (pp.865). However, they also recommended that "decisions regarding instituting or continuing therapy should be guided by the goal of improving the quality of life of the child and future adult" (pp. 865). This is curious given the report that clear relationships between GH treatment and psychosocial adaptation were not found in the research literature and, two pages earlier, had recommended psychologic or behavioral interventions should be used to treat issues of a psychosocial nature (22).

In 1997, the AAP Committee on Drugs and Committee on Bioethics published a statement on considerations related to the use of rhGH in children (4) that went beyond a summary of known physiologic effects of rhGH to include a substantial review of the literature on ethical issues, including a discussion of who is an appropriate candidate for treatment, the lack of established risk-benefit ratio, consideration of how treatment "success" is defined, elucidation of social justice issues, and an exploration of conflicts of interest. With regard to psychosocial issues in particular, the Committee recommended, "pediatricians need to exercise caution in administering GH therapy. Therapy may be justified for children whose height could prevent them from participating in the basic activities of daily living. Additional research is necessary to determine which of these children actually have increased final adult height, whether they actually experience a psychosocial benefit from any such increase, and whether GH therapy creates significant risks or other negative effects. The results of this research should provide clearer guidance on the future appropriate use of GH therapy" (pp. 127). Again, the guidance is a call for research.

Neither LWPES nor AAP guidelines from the 1980's and 90's stand alone in the focus on auxological and other physiological outcomes (both risks and benefits), yet intimating that rhGH treatment is helpful in addressing presumed issues of psychosocial adaptation and quality of life (QoL), and calling for research on psychosocial aspects of rhGH treatment. Similar admonitions are reflected in the 2003 American Association of Clinical Endocrinologists position statement (23), the LWPES update of guidelines (24) (both of which focused solely on somatic aspects) and the 2008

consensus statement on the diagnosis and treatment of children with ISS (25).

Most recently, a new approach was taken in issuing revised guidelines. The Pediatric Endocrine Society (PES; formerly LWPES) Drug and Therapeutics Committee and Ethics Committee issued a 2016 update to the 2003 guidelines titled "Growth Hormone and Insulin-Like Growth Factor-I Treatment in Children and Adolescents: Growth Hormone Deficiency, Idiopathic Short Stature, and Primary Insulin-Like Growth Factor-I Deficiency" (26). The guidelines followed the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach with regard to physiological effects of rhGH therapy. Psychosocial aspects of short stature and rhGH treatment were noted to have been recently systematically reviewed elsewhere (27) – thus not reviewed in depth within the updated guidelines. In a follow-up article summarizing the new PES guidelines, Grimberg and Allen (28) noted that "height is a poor predictor of psychosocial adaptation" and imply more research is needed to substantiate any psychological benefits of rhGH treatment.

Research Progress

To settle questions about the psychological risks and benefits of rhGH treatment, double-blinded trials in which patients with short stature are randomized to either rhGH or placebo arms and in which multi-informant (e.g. self-, parent-, and teacher-report) and multimethod (e.g. paper-and-pencil survey measures, direct observation, peer sociometry, and standardized testing results—including school-based assessments performed by qualified psychologists) outcome measures are needed. However, it is unlikely that a robustly designed randomized, placebo-controlled trial (RCT) can be conducted in the future. rhGH is widely available outside the context of clinical trials; as such, there is no incentive to participate in a trial where subjects may be randomized to placebo if everyone believes that rhGH will "help."

In point of fact, several research studies have been proposed or discontinued according to this belief. In 1989, the University of Nebraska Institutional Review Board concluded that the placebo control condition did not meet criteria for classification as "minimal risk," offered no direct benefits to participants, and would not yield information of "vital importance" to treat Turner syndrome (29). This decision was used by the Foundation on Economic Trends and the Physicians Committee for Responsible Medicine (PCRM) to voice concerns about rhGH treatment (subsequently termed "GH Experiments" on children with short stature by the PCRM) (30), and halt an NIH trial of rhGH in healthy short children that began in 1987 (31). By 1993, others similarly argued that daily injections of a placebo administered to children over the course of several years is unethical (32). Conversely, one might also successfully argue the case that daily injections of rhGH in healthy short children is unethical when there exists no clear evidence that it treats any medical or presumed psychological condition and the treatment may carry known and unknown risks (28).

Subsequent RCTs that made use of placebo control in the design were largely unsuccessful. In one, the control group was eliminated after parents of every single patient randomized to the control group refused participation since they could get rhGH elsewhere (33). In another, the monitoring board recommended study discontinuation "because the slow accrual of additional data did not warrant continuation of the long-term placebo injection control group." (34) In short, researchers found it virtually impossible to recruit participants. In sum, there have been repeated calls for high quality research on the psychological aspects of short stature and the effects of rhGH on a variety of psychological and psychosocial endpoints. As the following section details, strongly held negative stereotypes associated with short stature are pervasive. The dearth of rigorous research on psychosocial outcomes (35), in combination with beliefs that short stature constitutes a risk factor for healthy psychological development, has contributed to the current belief- rather than evidence-based rationale for rhGH treatment of short stature in youth who are GH-sufficient.

Beliefs about Psychosocial Aspects of Short Stature and rhGH Treatment

The concluding remarks of 1983 joint statement included the presumptions that those with short stature face handicaps, have problems of psychological adaptation, and hGH treatment can "significantly improve emotional status" (8). Examining these assumptions in turn, the literature reflects that negative stereotypes about the psychosocial adjustment of those with short stature are plentiful and have been reviewed extensively (36). In general, while empirical support for stereotypes is often found in analogue studies, limited support is found in population- or clinic-based studies (**table 1**, top panel) (20). Analogue research refers to studies in which simulations of real life are used - often including experimental manipulation of variables of interest. As analogue studies face criticism as being of questionable relevance to complicated social phenomena (37), population- and clinic-based studies rise in importance.

Focusing on clinically-referred children with short stature, it is often assumed that they experience chronic psychosocial stress due to their short stature that results in significant problems in psychosocial adjustment; it is further posited that increased growth velocity and/or height will ameliorate these issues. Evidence, however, demonstrates this is not the case (20); in

other words, psychosocial stress of the nature experienced by this population is not reflected in psychological or psychosocial dysfunction (38). Despite this, beliefs to the contrary persist not only in the general population, but extend to primary care and specialty physicians (39,40). In a national study of 727 pediatric endocrinologists' recommendations regarding initiating and discontinuing rhGH, more than 50% reported the emotional well-being of children and adults with heights less than the third percentile is "sometimes" impaired, and more than 25% responded it was "often" or "always" impaired (40).

Current Status of the Research Literature

An update of a 2016 systematic review of research (27) on the psychosocial adaptation, cognitive function, academic performance, and health-related quality of life (HRQoL) of youth with short stature treated with hGH/rhGH follows.

Materials and Methods

The methods in this update mirror those from the 2016 analysis, with 1 exception: to avoid redundancies in the literature evaluated, this review was restricted to studies published between 2014 to 2018; 2014 was included in the search to accommodate lag times in indexing. Studies represented in the earlier review were removed from current analyses. For a detailed explanation of the materials and methods used (27).

Study inclusion

Reports of studies in which rhGH was administered for treatment of short stature during childhood or adolescence were targeted. Studies were potentially eligible for inclusion if one or more psychological outcome variables (psychosocial adaptation, cognitive function, academic performance, or HRQoL) were measured.

Table 1. Stereotypes and assumptions related to short stature

Stereotypes	Analogue Study Findings	Population and Clinic-Based Study Findings
Children and adults with short stature are less psychosocially well adjusted	Generally supported	Not supported by population- or clinic-based studies
Children and adults with short stature are treated poorly due to their stature	Mixed results	Evidence of teasing and juvenilization from clinic-based studies
Short men are less attractive and desirable to women	Generally supported	Limited support in population-based studies: effect attenuated when statistically controlling for confounding variables
Children and adults with short stature do less well at school	Generally supported	Not supported by population- or clinic-based studies of children or adults
Adults with short stature hold lower status occupations and are paid less	Supported	Limited support in population-based studies: effect attenuated when statistically controlling for confounding variables
Assumptions	**Clinic-Based Study Findings**	
Patients with short stature experience chronic psychosocial stress	Supported	
Stature-related social stress results in significant psychosocial adjustment problems	Limited support: teasing and juvenilization were related to behavior problems; however, overall psychosocial adaptation was equivalent to community norms	
Patients with short stature exhibit clinically significant psychosocial adaptation problems	Not generally supported	
rhGH therapy induced increases in growth velocity and height result in improved QoL	Not supported	

QoL = quality of life; rhGH = recombinant human Growth Hormone
Table adapted from: Sandberg & Colsman 2005 (19)

Search and study selection methods

Studies with a publication date between 2014 to 2018 were included, after removing any studies already represented in the earlier review. Multiple electronic databases were searched using the following search string (and variations, as required by individual databases): "short stature" AND "GH" AND (behavior* OR "QOL" OR psych* OR cognit* OR academic OR education* OR intell*). Databases included: Cumulative Index of Nursing & Allied Health Literature, Ovid MEDLINE, PsycINFO, PubMed, Web of Science, and the Cochrane Database of Systematic Reviews. Finally, the reference sections of original research studies and reviews identified via electronic searches were assessed for additional references.

Two investigators (TS and MG) independently screened titles, abstracts, and keywords of all retrieved citations for evidence that the study included data on psychosocial adaptation, cognitive/academic performance, or HRQoL outcomes of those treated for short stature with rhGH during childhood or adolescence. Of those meeting criteria, the full text of each study was subsequently reviewed to ensure its eligibility.

Data extraction and management

Studies were independently evaluated by two investigators (TS and MG) who extracted data to a standardized coding sheet following recommendations of the Cochrane Collaborative pertaining to risk of bias in research design and reporting of findings (41). Ratings were subsequently compared across reviewers; disagreements were resolved through consensus involving a third independent reviewer (DES).

Assessment of risk of bias

The Cochrane Collaboration's Handbook for Systematic Reviews of Interventions provides a framework for critically evaluating the methodological quality of research; ie, the degree to which the study is free of bias (41). Risk of bias refers to the risk that a study overestimates or underestimates true intervention effects (42). Given that the specific categories of risk of bias vary across randomized and nonrandomized trials, separate assessments were conducted. For randomized trials, key domains assessed included: sequence generation (i.e. method by which participants are randomized to treatment or control condition), allocation concealment (i.e. method by which randomization is kept concealed, so that intervention allocations could not have been foreseen in advance of, or during, participant enrollment), blinding (i.e. masking treatment condition to the participants and study personnel so that knowledge of treatment does not influence outcome), incomplete outcome data explained (i.e. missing data due to exclusions by investigators or attrition by participants are explained), selective outcome reporting (i.e. failure to report all prespecified primary outcome data), and other potential sources of bias. For nonrandomized studies, risk of bias in controlling for confounding was substituted for sequence generation and allocation concealment. Additionally, participant diagnosis and whether or not authors listed the study as sponsored by the pharmaceutical industry were recorded. For all studies, a low, high, or unclear risk rating was made for each domain (41). A rating of "low risk" was assigned if the domain criterion was met and there was low risk of bias affecting the study's internal validity. A rating of "high risk" was made if the specific domain criterion was not met, thereby raising concerns over study validity. Risk of bias was considered "unclear" if there was insufficient information reported to determine whether domain criteria had been met or if there was not enough information to determine whether the noted risk would influence interpretability of results. In addition to rating individual domains, overall summary assessments were made for all studies. Overall risk of bias for each study was judged as follows: low, if there was a low risk of bias for all key domains; unclear, if an unclear risk of bias existed for one or more key domain, with the remainder of domains judged to be at low risk of bias; and high, if a high risk of bias existed for one or more key domains within a study. Industry sponsorship was recorded but was not included in this summary assessment score because it is not a domain considered in the assessment of the risk of bias according to the Cochrane Collaborative (41).

Results

Studies identified

Electronic literature searches returned 137 entries. Entries were excluded after a review of titles, abstracts, and keywords. Redundancies (additional versions of articles identified by more than one database, or articles included in the previous review) (27) were eliminated. At all times, reviews were geared toward overinclusion of studies; for example, when information was insufficient to judge appropriateness for inclusion, studies were retained. The remaining 18 entries were identified as potentially relevant and were selected for further review. Based upon this review, 17 articles were selected for inclusion in this report.

Diagnoses

Of studies reviewed, seven reported on the effects of rhGH in individuals with GHD, five with ISS, two for TS, three for SGA, seven for PWS, one for pediatric cancer, and one for multiple pituitary hormone deficiency. The sum of the diagnoses exceeds the total number of studies, given that two studies included more than one diagnostic group.

Outcomes

Many of the studies evaluated multiple outcomes separately. In this review, the risk of bias is evaluated separately for each

pertinent outcome address (HRQoL, psychosocial, cognitive, and academic). In this manner, 29 outcomes were identified across the 17 studies reviewed.

HRQoL and psychosocial outcomes (n=9 and n=10, respectively) were based on self-administered paper- and pencil questionnaires completed by the patient or parent, most of which were standardized on a normative population sample; however, some measures were authored by researchers for their studies, and normative data were unavailable for comparison. Cognitive function (n=9) was assessed using standardized batteries such as the Wechsler Intelligence Scale for Children, Wechsler Preschool and Primary Scale of Intelligence, Wechsler Adult Intelligence Scale, or other clinician administered neurocognitive tests. Academic performance (n =1) was assessed using the highest educational level attained.

Industry sponsorship

Overall 12 studies were industry sponsored (71%), and five were not (29%). Half (n=6) of the industry sponsored studies used a randomized design; the other half used nonrandom assignment. One (20%) non-industry sponsored study used a randomized design; 4 (80%) used nonrandom assignment.

Risk of bias

Of the 17 studies (with 29 outcomes) reviewed, risk of bias was rated as low for one study outcome (43), unclear in seven others, and high risk for the remaining 21 measured outcomes (**table 2**).

Table 2. Assessment of overall risk of bias per study outcome (n = 29)

	Risk of Bias Rating		
Study Design	Low	Unclear	High
Random	1	6	6
Non-Random	0	1	15
Total	1	7	21

In randomized studies, the most common risk of bias was a failure to blind participants (38%) and "other forms of bias" (69%). "Other forms of bias" in randomized studies included: measures lacking validity, the assessments used varied across sample, lack of statistical power, lack of a control group, lack of control for other medication given to the sample, and poor explanations for missing data. All randomized studies addressed incomplete data and were free of selective reporting (**figure 1** and **Supplemental table 1**). The sole randomized study exhibiting a low risk of bias for one of two psychological endpoints involved examination of cognitive performance and HRQoL in patients with PWS. The psychologist testing IQ was blinded to patient rhGH status, but HRQoL assessed by parent and self-report was not blinded in this open trail.

The most common risk of bias in nonrandomized studies included a failure to blind participants (94%), and failure to control for confounding variables (94%) (**figure 2** and **Supplemental table 2**). According to Cochrane Handbook for Systematic Reviews of Interventions, control for confounding in non-randomized studies includes: matching, stratification and modeling (41).

Discussion

Comparison to previous research

The earlier report by Gardner and colleagues (27) concluded that "The high risk of bias present in the majority of the literature on GH treatment effects on psychological outcomes (in particular, lack of blinding) substantially weakens confidence in their results." Findings from this review are concordant with previous results: lack of blinding remains a consistent source of bias in literature on rhGH treatment effects on psychological outcomes. Overall, most studies examined in the previous and the current review were rated as having a high risk of bias.

The Cochrane Handbook for Systematic Reviews of Interventions, acknowledges that the likely magnitude of bias associated with any domain varies (41). In the area of rhGH treatment effects, psychosocial adaptation and HRQoL are commonly assessed through subjective patient- and/or parent-report questionnaires. Although cognitive abilities are frequently assessed using standardized procedures performed by psychologists or psychometricians, knowledge of the person's treatment history can influence test results. Placebo control is of great importance in research where the outcomes are subjective and measured on a continuous scale; ie, the vast majority of psychosocial assessment measures adopted in this literature (44,45). It is likely that the risk of bias (especially in terms of placebo control and blinding) prevalent in the literature on the effects of rhGH on psychosocial outcomes will continue to compromise the results.

Persistence of Beliefs and Stereotypes Despite Contradictory Evidence

Given no evidence-based direct link between short stature and psychosocial adaptation or QoL concerns, nor one between rhGH treatment and psychosocial adaptation or QoL, how is it that statements regarding such putative associations continue to appear in clinical management guidelines? Both the "focusing

illusion" and the fact that height can serve as a proxy for health have been proposed to explain the disconnect between beliefs and evidence (46). According to Schkade and Kahneman (47), "When a judgment about an entire object or category is made with attention focused on a subset of that category, a focusing illusion is likely to occur, whereby the attended subset is overweighted relative to the unattended subset. In particular, when attention is drawn to the possibility of a change in any significant aspect of life, the perceived effect of this change on well-being is likely to be exaggerated." In other words, if stereotypes about the attributes associated with short stature are generally negative, it follows that QoL evaluations that focus on this isolated trait would be negative. Origins of the negative short stature stereotypes can be found in research under the rubric of evolutionary biology, which suggests that somatic characteristics that are correlated with physical health serve as "signals" of such and are perceived as attractive (48). The "Barker" or "fetal origins" hypothesis posits that prenatal experiences shape multiple health outcomes; eg, prenatal nutrition affects both stature and QoL adaptations (49). In other words, stature is a proxy (but not cause) of health and adaptation (50). Correlation and causation can be easily confused (37), particularly in light of the tendency toward confirmatory biases. Confirmatory biases refer to the tendency to seek or interpret evidence in ways that are partial to existing beliefs, expectations, or a hypothesis in hand (51). Research demonstrates people form opinions early, then evaluate subsequently acquired information in a way that is partial to that opinion – even in the face of compelling evidence that it is wrong (51,52).

A Way Forward: Shared Decision Making

Aside from cases where documented GHD exists, decisions for or against rhGH treatment in children are complex. They involve consideration of patient and family values balanced with a growing, but as yet incomplete, research literature on the predicted effects of GH as well as risks of the treatment. Recommended by the 2016 Guidelines for Growth Hormone and Insulin-Like Growth Factor-I Treatment (26), shared decision making (SDM) emerges as an avenue by which providers and patients/families can collaboratively arrive at clinical management decisions based on research evidence that balances risks and expected benefits with patient/family preferences and values (53,54). SDM requires recognizing that a decision is required, knowing and understanding the best available evidence, and incorporating the patient's (and, in pediatrics, the parents') values and preferences into the decision.

With regard to rhGH, there are two aspects of rhGH treatment to consider: the end goal and the process by which this is achieved. First, the process of treatment begins with informed consent, or technically "informed permission" in the case of parents or other surrogates with child's assent (55), where appropriate. Height gains from rhGH treatment in children with chronic renal insufficiency are approximately 3 to 9 cm (10), 5 to 8 cm in Turner syndrome (11), 18 to 24 cm in PWS (12), 6 cm in SGA (13), 3 to 7 cm in ISS (14,15,56), 8 cm in SHOX deficiency (16), and 4 to 14 cm in Noonan syndrome (17). Patients and families should be informed of this and consider how important those gains are in relation to risks, known and unknown. Information on safety in the near and long-term on physical health is one aspect. Another involves "treatment burden" or "spillover effects" (57) (i.e. the act of daily injections and regular visits to endocrinology specialists that emphasize growth may negatively affect patients' self-perceptions) (58). Additional practical considerations include travel costs, time away from school and jobs, the route of treatment and out-of-pocket costs - including co-pays and deductibles for the rhGH itself, treatment monitoring tests (labs, bone ages), and clinic visits (59).

The final phases of the SDM process require examining the patient's and family's expectations about what rhGH treatment will do for the child and the degree to which the evidence fits with the expectations and values of the patient, the family, and of your own and other members of the youth's healthcare team - doctors bring their own biases to the discussion (39,40). Presumably, they intend it to increase height, but by how much? And what will that increase in height do for the child physically, psychologically, socially, cognitively, academically or vocationally? (59) Explore with the family what the research literature shows about each of their expectations as well as your own expectations with an eye on seeking information that counters unrealistic expectations and false beliefs.

Conclusion

Between 1958 and the present, dramatic changes have occurred in the clinical management of short stature using GH. Initially, the limited supply of pituitary-derived hGH dictated strict criteria for diagnosing GHD – the only condition for which hGH was approved as a treatment – and tightly controlled treatment protocols in which patients were de facto research participants. The focus of research was on auxological outcomes with careful monitoring of risks to health and safety. With the advent of biosynthetic rhGH, the supply increased, the number of indications for treatment has grown, and the focus of intervention changed from hormone replacement to treating short stature itself. As embodied in the 1983 joint LWPES / AAP statement, improved psychosocial adaptation was also an underlying, albeit largely unspoken and unexamined target of treatment.

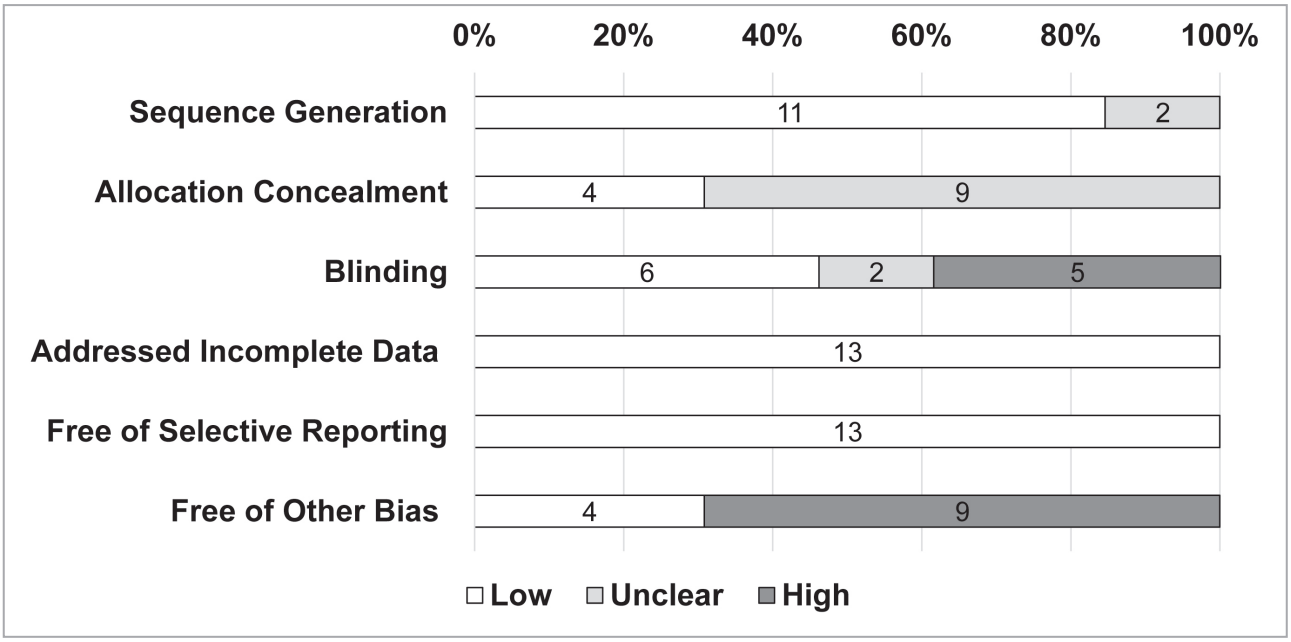

Figure 1. Risk of bias summary: Randomized study outcomes (n=13)

Supplemental Table 1. Risk of bias in outcomes (n=13) measured in randomized studies (n=7)

First Author, Year	Diagnoses	Outcomes Measured	Cochrane Review Criteria: Risk of Bias						Industry Sponsorship Listed
			Sequence Generation	Allocation Concealment	Blinding	Incomplete Data Addressed	Free of Selective Reporting	Free of Other Bias	
Bakker, 2015	PWS	Cog	Low	Unclear	Low	Low	Low	Low	Yes
		HRQoL	Low	Unclear	High	Low	Low	Low	Yes
Bohm, 2015	PWS	Cog	Unclear	Unclear	High	Low	Low	High	No
		Psy	Unclear	Unclear	High	Low	Low	High	No
De Schepper, 2016	SGA	Cog	Low	Unclear	Unclear	Low	Low	High	Yes
		Psy	Low	Unclear	Unclear	Low	Low	High	Yes
Freriks, 2015	TS	Cog	Low	Unclear	Low	Low	Low	High	Yes
		HRQoL	Low	Unclear	Low	Low	Low	High	Yes
		Psy	Low	Unclear	Low	Low	Low	High	Yes
Kuppens, 2016	PWS	Cog	Low	Low	Low	Low	Low	High	Yes
Lo, Festen, 2015	PWS	Cog	Low	Low	Low	Low	Low	Low	Yes
		Psy	Low	Low	High	Low	Low	Low	Yes
Lo, Siemensma, 2015	PWS	Psy	Low	Low	High	Low	Low	High	Yes

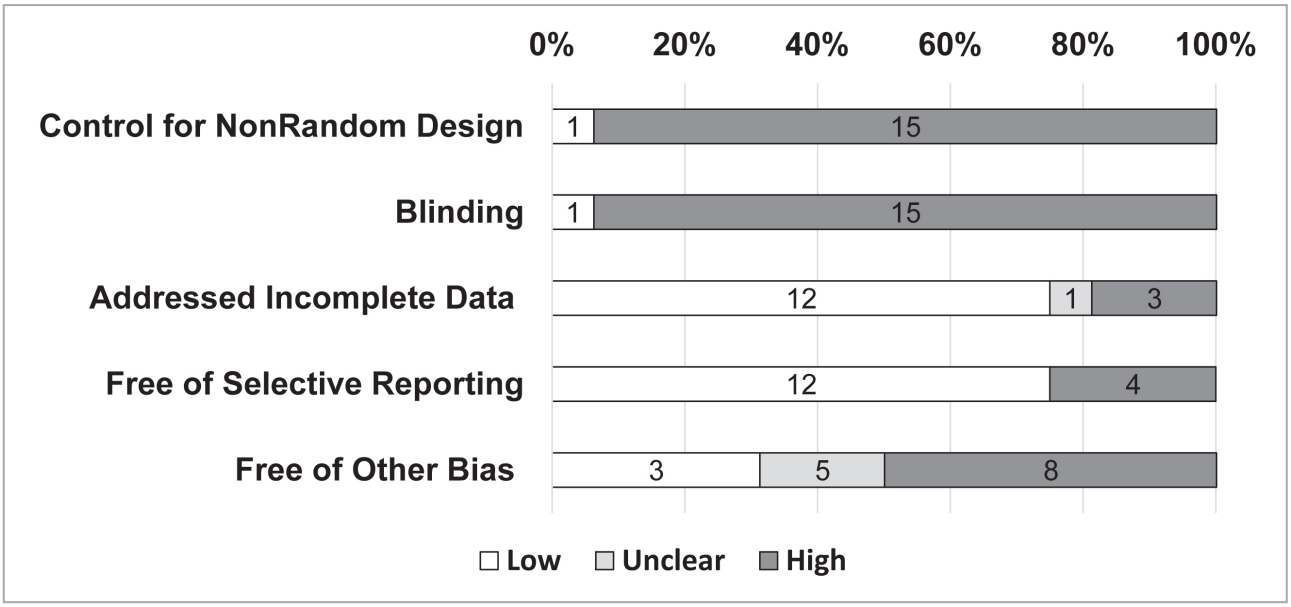

Figure 2. Risk of bias summary: Non-randomized study outcomes (n=16)

Supplemental Table 2. Risk of bias in outcomes (n=16) measured in non-randomized studies (n=10)

First Author, Year	Diagnoses	Outcomes Measured	Cochrane Review Criteria: Risk of Bias					Industry Sponsorship Listed
			Control for Non-Random Design	Blinding	Incomplete Data Addressed	Free of Selective Reporting	Free of Other Bias	
Brod, 2017	GHD	HRQoL	High	High	Low	High	High	Yes
		Psy	High	High	Low	High	High	Yes
Chaplin, 2015	ISS, GHD	Cog	Low	Low	Low	Low	Unclear	Yes
Dykens, 2017	PWS	Cog	High	High	Low	Low	Low	No
		Psy	High	High	Low	Low	Low	No
Ludwig, 2016	PWS	Cog	High	High	High	High	High	No
		HRQoL	High	High	High	High	High	No
Oświęcimska, 2014	GHD	HRQoL	High	High	Low	Low	High	No
Quitmann, 2016	ISS, GHD	HRQoL	High	High	Low	Low	Unclear	Yes
		Psy	High	High	Low	Low	Unclear	Yes
Silva, 2017	ISS, GHD	HRQoL	High	High	Low	Low	Unclear	Yes
		Psy	High	High	Low	Low	Unclear	Yes
Sommer, 2015	ISS, GHD, TS, SGA, Other	Aca	High	High	Low	Low	High	Yes
		HRQoL	High	High	Low	Low	High	Yes
Takahashi, 2017	SGA	Psy	High	High	High	Low	High	No
Tanaka, 2014	SGA	HRQoL	High	High	High	Low	Low	Yes

In general, and in contrast to prevailing beliefs about the psychosocial adaptation of those with short stature, research on the psychosocial adaptation of those with short stature has shown mixed results. Analog studies (e.g. where study participants are asked to relate height with success in life) tend to support the notion that taller is better and shorter is worse. However, those studies conducted in real-life and with attention to selection biases (i.e. population-based studies or those conducted in clinics) do not generally support the notion that those with short stature experience significant problems of psychosocial adaptation. Complicating the ability to make a definitive statement on the effects of rhGH on psychosocial adaptation is the quality of the literature. The high risk of bias present in the majority of the literature on rhGH treatment effects on psychological outcomes (in particular, lack of blinding) substantially weakens confidence in their results. While this may serve to explain the variability of findings regarding psychosocial outcomes across studies, it means that questions cannot immediately be answered. Conducting a placebo-controlled RCT is made extraordinarily difficult (if not impossible) at this time, yet studies have been improving over time. Guidance offered by professional societies have formally and increasingly taken note of psychosocial and ethical issues since the 1983 statement.

This paper hopefully clarifies that in the field of short stature and GH treatment, increased growth velocity and adult height are proxy measures for the implicit, but undoubtedly the primary outcome: psychosocial adaptation and QoL. What is needed are studies that convincingly demonstrate, through rigorous research design and methodology, that the benefits of rhGH exceed the risks and burdens.

Disclosure

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rhGH Abuse for Sports Performance

Alan D. Rogol, MD, PhD

Professor, Emeritus, Department of Pediatrics, University of Virginia, Charlottesville, VA, USA

Corresponding author: Alan D. Rogol, MD, PhD, Department of Pediatrics, University of Virginia, Charlottesville, VA, USA, E-mail: adrogol@comcast.net

Abstract

Doping is at least as old as the ancient Olympics. Substances taken to improve athletic performance ranged from stimulants to hallucinogenic plant substances, but more recently include anabolic agents. Recombinant human growth hormone, rhGH, is one agent with a relatively short history of use, but few data to unequivocally show that it actually improves performance. However, rhGH has therapeutic use for those GH deficient and the concept of a therapeutic use exemption for those with documented deficiency is outlined along with doping control methods.

The athlete's biological passport, a document with all of the analytical data from an athlete, helps in doping control because any one individual will vary for any analyte over a more narrow range than that for a "normal" control population.

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Introduction

"Doping" is as old as the ancient Olympics (776 BC to 394 AD) when athletes used herbal medications, animal organs (organo-therapy was common to cure diseases and to improve vitality), stimulants, and hallucinogenic mushrooms to improve athletic performance. At that time and for many centuries it was not considered illegal or even unethical (1). It was likely standard practice at the end of the 19th and into the 20th century and carried out openly. Dr. Otto Rieser not only spoke out about "Doping and Doping Substances" in 1933, he noted the part that physicians played (knowingly or not) by prescribing potent drugs for the purpose of sport performance (quoted in 2). The anabolic steroid era began in the 1950's (1) and consistent hGH use likely in the 1980's, when the recombinant hormone became available. Given the prohibitions of the World Anti-Doping Agency (WADA) and most major athletic associations it is unlikely that we shall ever know the specific effects of doping level doses of rhGH on human physiology or athletic performance.

The field of GH and its related compounds is only getting broader with the advent of long-acting GH molecules likely to be available in the next few years and analogs of IGF-I, including mechano-growth factor (synthetic peptide) (3) and a host of GH-releasing peptides. Athletes will continue to use them and try to stay ahead of the analytical chemists (doping control), irrespective of whether there are compelling data that administering any of these compounds improves the desired endpoint—athletic performance.

Growth Hormone and Physical Activity (Performance)

Aerobic exercise is a well-recognized stimulus to growth hormone secretion. In some clinics it has been used as a screening test for GH sufficiency, but its lack of standardization and modulation by many factors including, age, stage of pubertal maturation, previous food consumption, relative fatness, and likely a host of other factors has generally precluded its use as a standardized "stimulation" test. Quantitatively, this has been studied in children (bicycle ergometer) (4), employing a graded series of exercise intensities (5), following repeated bouts of exercise (treadmill and bicycle ergometer) (6), and following more than one

year of progressive endurance training (7). These data may be summarized to indicate that: "intense enough" exercise is a strong stimulus to GH release in children and adults, the release is intensity dependent, that a year of endurance training amplifies the pulsatile release of GH, especially when some of the training occurs above the individually determined lactate threshold, and that repeated bouts of exercise within the same day whether sequential or delayed across the day increase the daytime output of GH compared to the no exercise state.

Growth Hormone Deficiency

Growth hormone is important for the maintenance of body composition and physical performance. Those with GH deficiency show: decreased lean body (muscle) mass, strength, increased body fat mass with a more central distribution; reduced maximal oxygen uptake and cardiovascular function (decreased exercise performance); increased cardiovascular risk factors and diminished quality of life (8). Most of these deficiencies are ameliorated (normalized) with proper replacement therapy with rhGH, although that may take three years and more of replacement therapy to restore structure and function but to a lesser degree than normal for age (9,10). Stopping replacement therapy abrogates most of these gains (11).

Growth Hormone Excess (Acromegaly)

Clinical features of acromegaly include local tumor effects, somatic effects including virtually all systems and major effects on carbohydrate metabolism, as noted in detail (12). Relentless overproduction of GH over many years leads to larger, but functionally deficient, muscles, arthralgia and arthritis, and reduced body fat with disordered regional distribution. In normal cattle GH "therapy" is considered a partitioning agent to divert dietary calories into lean body mass (protein synthesis) and away from fat synthesis (13). This concept is magnified in animals made transgenic for GH that have far greater lean body mass and much less body fat, similar to the situation in patients with acromegaly (reviewed in 14).

Recombinant Growth Hormone for Performance (and Body Image) Enhancement

The sections reviewed above are mere prologue to the issue of whether doping (that is, generically using artificial means to enhance athletic performance; or, more practically, administering non-medically required substances in supraphysiologic amounts or using a banned method, for example, blood transfusions, to attempt to enhance appearance or performance) is actually effective for either recreational or elite athletes. From the data reviewing replacement therapy in children and adults with documented GH deficiency, one easily notes amelioration of most of the symptoms and a recrudescence of them months after ceasing to take the agent (*vide supra*). Thus, the rationale is that if the physiologic amount can make performance better, then supraphysiologic amounts of exogenous hormone might make those with physiologic amounts of endogenous hormone perform better as well. It should be remembered that because of feedback auto-inhibition, exactly replacing the physiologic amount of GH should engender no benefit, since the body would be exposed only to the equivalent physiologic amount, although in a non-physiologic (non-pulsatile release) pattern.

What does the athlete expect to attain with exogenous rhGH? Leaving aside the issue of dose, for one "knows well" the replacement dose, but really does not know the "doping dose", which may be four to ten or more times that. The expected effects with doping are likely to be the enhanced physiologic ones:

1. Body composition: decreased fat mass, increased lean body mass (with the caveat that some of the increase is in extracellular water), and increased bone mineral density.
2. Physical appearance and performance: more muscular look with diminished fat—the "ripped" look favored by body builders; increased strength, maximal oxygen consumption and cardiovascular function.
3. Altered metabolism (complex): with alterations in carbohydrate and lipid metabolism to "fuel" the muscles and cardiovascular system (including a minor increase in hematocrit) to elicit the salutary effects on performance (15).
4. To shorten the recovery time following exhaustive training or recovery from musculoskeletal injury.

What is the evidence that administering supraphysiologic amounts of rhGH will enhance performance? There are virtually no studies in elite athletes in whom an even miniscule change in performance might change the order of finish in an athletic event. The majority of studies are in relatively small numbers of recreational athletes who are receiving known amounts of therapeutic-grade drug. That is likely to be very different from those who are doping with rhGH, not only in the amount but also in that rhGH is but one of a "cocktail" of illicit (in the athletic sense) substances administered to elite athletes. The primary "other" substance, at least for males, is androgenic steroids. A number of investigators have studied both substances in older individuals and in some recreational athletes and it may be difficult to disentangle the drug interactions.

One may begin with a systematic review (it is not a meta-analysis) of the few studies in young recreationally active athletes. Liu and colleagues reviewed a series of reports of recreational athletes receiving rhGH of variable duration (16). The weight lifting activities continued during these trials. Their analysis noted a small, but overall non-significant decrease in fat mass. Their overall conclusion was that using relatively low doses (but mostly supraphysiologic) led to increases in lean body mass, but not more body strength in either the upper or lower extremities. Much of that resides in the extracellular compartment (water) and is not part of the body cell mass. This is consistent with the anti-natriuretic effects of rhGH, especially as administration is commenced. It is this property that likely leads to the edema and arthralgia noted in patients just beginning replacement therapy or in those with acromegaly (17). There was either no change or a small decrement in aerobic exercise, especially noting that a similar work load led to higher lactate levels when receiving rhGH.

Several studies require more in depth analysis. Yaresheski and co-workers studied both experienced weight lifting athletes and untrained young men before and after 2 weeks of a large dose (40 µg/kg, daily) of rhGH (18,19). This was enough to more than double the circulating IGF-I concentration into the abnormally high range as an indication of the "excessive" dose. They measured the fractional rate of muscle protein synthesis and found no differences in the rate of synthesis or breakdown. Their interpretation from this very short experiment was that it would be unlikely that rhGH, alone, would lead to increases in muscle mass (not lean body mass) and strength in the longer term.

In an elegant randomized, placebo-controlled, blinded clinical trial of 8 weeks with 6 weeks washout and restudy after that, Meinhardt and colleagues evaluated the effects of rhGH alone or with testosterone on body composition and measures of performance in 96 recreationally-active adults (63 men; 33 women) (20). Men and women were randomly assigned to rhGH or placebo and some of the men to testosterone, rhGH or both testosterone and rhGH. The main outcome measures were changes in body composition, physical performance [extrapolated (from a population-based nomogram), V_{O2max}, strength (dead lift), power (jump height) and sprint capacity (Wingate test of anaerobic power)]. As an independent measure of efficacy, the investigators noted an approximately doubling of the circulating level of IGF-I. The principal results showed that body cell mass was correlated with each measure of performance at baseline. Body composition variables showed a decrease in fat mass and an increase in lean body mass mainly due to an increase in extracellular water. There was an increase in body cell mass in those men receiving testosterone as well as rhGH. For the performance measures rhGH significantly increased "sprint capacity", that is showed an increase in anaerobic power output by approximately 4% in men and women, but by more than 8% in the men in the combined administration group. The increase in anaerobic power was not maintained after the 6 week washout period. These anabolic drugs had no effect on the aerobic capacity or any measure of strength or power. These data indicate clear changes in body composition with the dose of rhGH likely 3-fold higher than replacement dose in adult men and perhaps 2-fold higher in estrogen-replete women. How might these data be interpreted quantitatively in terms of athletic performance? The investigators themselves called the change in performance in the Wingate test as an augmentation of "sprint capacity" and made a "theoretical" calculation (based on mechanistic and conceptual extrapolation) of how that might change one's performance in an anaerobic test, for example, diminution of one's time to complete the 100 m dash. Therein lies the problem. There are so many vagaries in addition to anaerobic power that may affect performance at a given time for a given task. As noted previously the dose taken by athletes to enhance performance is likely greater and is often combined with other anabolic agents. The "usual" combination is with testosterone and it is that group that had the greatest body composition and performance changes in this clinical trial.

In another trial Graham and colleagues evaluated the effects of 6 day administration of rhGH (at a relatively high dose compared to replacement dose, i.e. 58 µg/kg per day) on a group of former anabolic steroid (ab) users (21). IGF-I levels rose as an indicator of biological effect. There were minimal but significant changes in body composition and significant increases in strength and peak power output. There are however several cautions in the interpretation of these data. The first is that the men are likely in a catabolic phase as they withdraw from their anabolic steroid use and that the pharmacodynamic effects of the anabolic steroids may have left the muscle in a particularly sensitive state to another anabolic agent, in this case rhGH.

How might rhGH work in athletes, elite or not? Given the evidence presented above one might believe that there was little chance of efficacy especially in the elite athlete. Perhaps the enhancement of performance relies on the anti-catabolic effects allowing the athlete to train more intensely and recover more quickly (22). In skeletal muscle and tendon the extracellular matrix confers important tensile properties. It is critically important for tissue repair after injury (23). Growth hormone is anabolic for connective tissue formation and increases circulating markers of collagen turnover (24). Studies in animals show that tendon injuries heal more rapidly with IGF-I therapy, an "intentional" consequence of GH administration (25). Doessing and co-workers investigated the 14 day administration of a supraphysiogic dose of rhGH (33-50 µg $kg^{-1}da^{-1}$) to healthy young men (23). Increased matrix collagen synthesis in skeletal muscle and tendon was noted

at 2 weeks. IGF-I levels rose approximately 3-fold to the supraphysiologic range. These investigators measured tendon collagen I mRNA and tendon collagen protein synthesis. They noted 3.9- and 1.3-fold increases, respectively. For the muscle tissue the increases in collagen protein synthesis were 2.3- and 5.8-fold the basal levels. However, protein synthesis in the myofibril was unaffected by the increased circulating levels of GH or IGF-I. Moderate exercise did not alter these findings. Overall, the results strongly suggest that it is the matrix tissue rather than the muscle fibrillary hypertrophy that is responsive to GH (and IGF-I) in the adult human musculotendinous tissue. They concluded that the GH/IGF-I system is more biologically relevant to regulate the supportive matrix in musculoskeletal tissue than it is for the myofibril-myocyte itself (23).

In virtually all of the trials of rhGH in adults, investigators noted improved quality of life as measured by a number of psychological inventories (26). Might the same be true for those recreational or elite athletes? Here the issue likely is inextricably entangled with the placebo effect, for the latter has been shown to increase physical performance and reduce muscle fatigue in properly designed trials (27).

IGF-I and GH Secretagogues

Theoretically, IGF-I and GH secretagogues should be anabolic and thus at risk to be abused by athletes. Their use (forms and amounts) in the athletic world is not well documented, especially the dosing and timing of doses. There are no convincing studies (clinical trials) to show ergogenic effects (28). Both should differ from GH, itself. First, IGF-I administered in amounts that might be equivalent to "doping" doses of rhGH would very likely cause significant, immediate hypoglycemia and based on some of the clinical trials in children be prone to induce intracranial hypertension. It would synergize with the early insulin-like effects of GH, but antagonize the later insulin antagonistic effects. In abrogating endogenous GH release the GH effects on lipid metabolism would vanish, and perhaps exacerbate an energy rich (lipid oxidation) process, so important to endurance exercise.

The multitude of GH secretagogues pose a different problem. After initial release of GH following the administration of GHRH, for example, the pituitary is at first absolutely refractory and then partially refractory to another release of GH for which somatostatin is at least partially responsible. Thus after the initial release of GH, somatostatin ought to dampen the continuing release of GH. Can this system be overridden? It is likely that it can, since those with GHRH secreting tumors often have acromegaly (29), indicating escape from physiologic regulation (30). Similar constraints follow the infusion of GHRH and GHRP-2 (31) and likely many other GH secretagogues.

Adverse Events

Adverse events for patients receiving replacement doses of rhGH have been extensively reviewed (17). The majority of effects involve fluid retention, myalgia and arthralgia. GH reduces visceral fat and increases lean body mass. The cardio-vascular risk (markers) which are adverse in adults with GH deficiency revert with rhGH therapy; however it is indeterminate that replacement therapy decreases the rate of cardiovascular events (17).

For athletes receiving higher doses (average dose 40 μg kg^{-1} da^{-1}) there are few studies, and all are short term. The adverse events center on fluid retention and include edema, arthralgia, and paresthesia in the extremities and perhaps some increased sweating (32).

There do not appear to be enough data for athletes receiving high doses of IGF-I. One might consider those from the clinical trials in humans with IGF-I deficiency: hypoglycemia, edema, and intracranial hypertension.

Doping Control

Endogenous GH is comprised of a number of isoforms with the major ones being 22 kD (~50%) and 20kD (5-10%). The rest is a series of dimers and oligomers and various sulfated and phosphorylated fragments (33). Pharmacologic grade rhGH is virtually 100% the 22kD isoform. The direct or isoform test takes advantage of that distribution and the pharmacologic blockade of pituitary GH secretion when the exogenous, 22kD form is administered. The circulating form is virtually completely the administered form. The test uses two sets of antibodies, one that is specific for the 22 kD form, and the other that measures most all of the circulating forms. The analytical finding is shown as the ratio of 22 kD to "rest" of the isoforms. The physiological ratio averages about 0.8 and various assays have specific "cut-points" (34). With this test the window for detectability is approximately 24 to 36 h. Given these assay characteristic one must strongly consider "out of competition" testing. The physiologic precept is that exogenous GH will shut off spontaneous secretion from the pituitary. The exogenous protein is rapidly cleared. If one has normal pituitary function, spontaneous release will resume within 48 hours of the last dose of rhGH (35,36).

A second (direct) isoform test takes advantage of a fairly constant ratio of the 20 kD RNA splice variant (lacking residues 32 to 46 of the full length 22 kD form) to the more prevalent 22 kD form (37,38). Its biological activity is similar to the longer form. Its utility for doping detection is that the ratio of 20 kD/22 kD changes quickly when rhGH (100 % 22kD) is administered. The test has excellent specificity, but very short

window of opportunity to detect the pituitary down regulation of the 20 kD form from the exogenously administered rhGH (39).

The indirect or biomarkers test measures circulating analytes that are affected by GH action. At present these are most commonly IGF-I and procollagen type III, amino-terminal propeptide (P-III-NP) (35). With this test the window for detectability is one to a few weeks. This test was introduced at the 2012 Olympic and Paralympic Games in London. Two Russian powerlifters, Nikolay Marfin and Vadim Rakitin had adverse analytical findings using this test. Both admitted to taking rhGH (40).

The standard tests for doping control for rhGH have been available for a number of years and have been periodically updated. In depth reviews with the pros and cons are available (34,35,41). There is no specific test for IGF-I except for measuring it directly, but that is the same analyte as the endogenous substance. Using the markers test for rhGH has also proved unsatisfactory because the rise in the level of P-III-NP is much reduced compared to that noted after rhGH administration (39).

After testing more than 1,000 samples, the first adverse analytical finding (positive test) was determined in February, 2010 in the British rugby player, Terry Newton whose positive test sample was obtained out of competition (35,42). Others found to have a similar adverse analytical finding include: Matt Soocholotiuk, a University of Waterloo (Canada) football player (43). American baseball soon followed when Mike Jacobs was suspended for 50 games in 2011 (44). Perhaps the most prominent case was that of Andrus Veerpalu, an Estonian Olympic gold medal winner in skiing. He challenged the adverse analytic finding to the Court of Arbitration for Sport and subsequently had the finding reversed because the court was not convinced that the threshold for considering finding was sufficiently reliable to uphold the doping conviction (45). An odd quote from the Court noted "that there are many factors in this case which tend to indicate that the Athlete did in fact himself administer exogenous hGH" (46). I am not sure how to interpret these seemingly disparate statements.

The WADA-approved labs processed approximately 20,000 tests for the years 2015 and 2016. Ten adverse analytical findings were detected or approximately 0.05%, most from out of competition testing. There were more athletes sanctioned for non-analytic reasons—some had skipped or not been found for 3 scheduled tests; others were in possession of, trafficked in, or in some other way connected to prohibited peptides and accepted 2 to 8 year sanctions (47). It should be remembered that these results only refer to the sporting federations that are governed by WADA rules. Many more amateur and professional leagues either have their own "mechanism" for determining which drugs, if any, to include on a banned list. More important is the overall program, pre-analytic and analytic, to detect these banned substances.

Doping Control, the Future

Given the inadequacies of the extant tests, others are being devised to broaden the duration of detection. These include a series of collagen-dependent peptides, and proteomic, nucleic acid and genomic-based strategies. Some of the limitations of the marker method include age of the athlete and gender dependency; therefore new techniques have been sought including a new set of biomarkers that would be reliable, robust and sensitive to permit longer term detection of rhGH (and perhaps IGF-I) doping. Ferro and co-workers have suggested FN1 and *RAB31* genes. The former is involved in GH action on collagens (48). The latter encodes a small GTP-binding protein (RAB31) that plays a role in vesicle and granule targeting (49). Likely because of IGF-I increases following the administration of rhGH these genes are activated and the protein produced. Preliminary data in plasma show statistically significant increases in the levels of FN1 for at least 9 days following daily administration of rhGH (26 $\mu g\ kg^{-1}\ da^{-1}$, a modest doping dose) for 3 days to 10 normal volunteer men (4 additional men received placebo injections) (48,49).

A further proteomics approach searching for novel biomarkers associated with rhGH administration to non-elite athletes showed promising results for a small number of individual proteins (50,51). A transcriptome approach was suggested by Mitchell *et al* who evaluated up- and down-regulation of genes within peripheral blood lymphocytes, but could not find quantitatively significant alterations (52).

Further approaches that are being developed include: use of hydrogel nanoparticles in a pre-possessing step to capture rhGH in the urine followed by isoform differential immunoassays as noted above (53). Another approach in the developmental stage is to use nanobodies, tiny antigen-binding molecules derived from camel heavy-chain antibodies and made into the usual sandwich ELISA configuration on the tip of M13-phage (54). Using a strategy similar to that for proteomics, Kelly and co-workers evaluated specific inhibitory RNAs comparing pre- and post-treatment cell-free samples following administration of rhGH (55). They identified (microarray) and confirmed (RT-qPCR) 4 miRNAs that were differentially expressed in individuals receiving therapeutic doses of rhGH.

Theoretically, these changes should also apply to some of the changes induced by administration of IGF-I. A different approach might be appropriate for IGF-I and GHRH analogs because each would have amino acids that differ from the native protein. The GH secretagogues might be traceable in the urine and would present many new compounds for a

urinary "doping" library using liquid chromatography followed by mass spectrometry (56).

Therapeutic Use Exemption

Some athletes will have conditions (or diseases) for which a WADA-banned substance (or method) is required to return an athlete to "physiologic" function (47). A common example in endocrinology would be insulin therapy for someone with type 1 diabetes mellitus. For these athletes a therapeutic use exemption (TUE) is available. It is for the use of a WADA-prohibited substance or method (for example, large volume intravenous infusion) to maintain the health of a competing athlete. The substance or method is required:

- To treat an acute or chronic condition
- Treatment return an athlete to normal health without offering any performance enhancement
- It is necessary because there are no other, non-banned alternatives
- Any previous therapies were ineffective
- It is not required as a consequence of prior (non-therapeutic) use of a prohibited substance.

The components of a TUE:

- Complete medical details including history, clinical findings and investigations
- The necessity to administer prohibited medications, including dosage, route and frequency of administration. These must be certified by a suitably-qualified medical specialist
- The medical necessity cannot be the result, wholly or partially, of prior use of a drug (or method) from banned classes or methods
- Under no circumstances will permission be given to use any synthetic anabolic steroid

For rhGH, anabolic steroids and insulin the suitably-qualified medical specialist is usually a board-certified medical or pediatric endocrinologist.

Athlete's Biological Passport (ABP)

The athlete's biological passport infers the use of a prohibited substance (or method) by the monitoring of discriminant biomarkers over time—likened to the longitudinal record of disease-related biomarkers for personalized medicine. It is operationally defined as the longitudinal profile and all other relevant information including training, competitions and information derived from investigations. The rationale for the longitudinal record is that doping substances trigger physiologic changes that provide physiologic enhancements. That is, doping can be detected specifically with selected biomarkers and like the differences between pharmacokinetics and pharmacodynamics, the biological effects of the drug remain detectable much longer than the substance (chemical) itself. Thus, there is a paradigm shift in doping control: from the direct identification of the banned substance to detection of abnormalities in biomarkers. The first module was constructed to detect blood manipulation by the use of erythropoietic-stimulating agents or via blood transfusion. The second, a steroid module to identify exogenous anabolic steroids or other indirect steroid doping substances or methods. A third module to detect use of prohibited peptides and protein hormones (for example, rhGH, IGF-I, insulin and rhGH-releasing peptides) is under study. The goal is to have one passport for each athlete with all data points included. Those data can come from any of the WADA-accredited and approved laboratories and are entered into a central database. An adaptive model using a Baysian-type of statistical analysis is applied to the longitudinal profiles and the appropriate agencies are notified when the profile is considered atypical.

Denouement

Virtually nothing other than the degree of sophistication is new about doping in sport. The availability of anabolic substances such as rhGH has broadened the perspective (and use) by athletes, despite the absence of compelling data for efficacy or even safety toward the desired outcome—enhanced athletic performance.

We as physician-endocrinologists, the "suitably qualified medical specialist," are required to learn and understand the rationale and process for a TUE as well as the purpose of and the data within an athlete's biological passport.

Disclosure

The author declares no conflicts of interest in the preparation of this manuscript.

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Monitoring rhGH Safety: rhGH Registries, SAGhE and Future Needs

Bradley S. Miller[1], MD, PhD, Ron G. Rosenfeld[2], MD

[1]Division of Endocrinology, Department of Pediatrics, University of Minnesota Masonic Children's Hospital, Minneapolis, Minnesota, USA, [2]Oregon Health and Science University, Portland, Oregon, USA, STAT5, LLC, United States

Corresponding author: Bradley S. Miller, MD, PhD, University of Minnesota Masonic Children's Hospital, Department of Pediatrics, Division of Endocrinology, 8952D, East Bldg MB671, 2450 Riverside Avenue, Minneapolis, MN 55454, Tel: 612-624-5409, Fax: 612-626-5262, E-mail: mille685@umn.edu

Abstract

The safety of growth hormone (GH) therapy in children has been studied extensively. The identification of Creutzfeldt-Jacob disease in individuals who received pituitary-derived GH led to heightened surveillance for safety issues related to recombinant human GH (rhGH). An excellent safety profile of rhGH has been demonstrated in large Phase IV registries comprising > 600,000 patient-years of rhGH exposure and long-term safety cohorts of adults treated with GH as children. Increased mortality risk has been reported but eliminated when corrected for small size at birth. Increased risk of mortality from cerebrovascular disease has been reported but interpretation of these events remains difficult due to the lack of appropriate control groups and a lack of replication of these findings in other studies. The advent of new long-acting growth hormone (LAGH) products provides an opportunity for the development of cohorts of individuals receiving LAGH replacement therapy for continued long-term safety studies.

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Introduction

Growth hormone (GH) was first purified from the bovine pituitary in 1945 and human pituitary in 1956. The first attempt at GH treatment of humans occurred in 1956 using bovine GH in a 3 year-old with presumed GH deficiency (GHD). Bovine GH was found to have no impact in humans (1). Human GH has been used to treat patients with GHD since 1957 beginning with GH extracted from the pituitary glands of human cadavers (pitGH) (2-4). Due to the limited supply of pitGH, the National Pituitary Agency (NPA) was developed in 1961 to collect and distribute pitGH for use in patients participating in clinical research protocols. In 1977, a centralized laboratory was established to extract pitGH. However, pitGH remained in short supply. pitGH was dosed based upon body weight and injected intramuscularly three times per week. Due to the continued short supply of pitGH, patients frequently went without pitGH for multiple months each year.

In 1985, three cases of Creutzfeldt-Jakob Disease (CJD) were identified and attributed to pitGH (1). The first case of this rare neurodegenerative disease was in a 22 year-old male who had received pitGH. When two other cases were identified in the US later that year, it was found to be associated with pitGH. The risk of this prion-transmitted disease was

associated with the method of purification of pitGH from cadaveric pituitary glands. At least 15 cases of CJD were identified in the US with more than 100 cases reported in France (5,6).

The same year that CJD was first identified, recombinant human growth hormone (rhGH) was approved by the US Food and Drug Administration (FDA). The availability of an unlimited supply of rhGH improved access to therapy for children with GHD, and the dosing frequency increased from three times each week to six or seven days each week initially via intramuscular and subsequently via subcutaneous injection (7). The increased supply of rhGH allowed investigation into treatment of multiple conditions associated with short stature not associated with GHD leading to FDA-approval for treatment of children with growth failure associated with chronic renal insufficiency (1993), Turner Syndrome (TS, 1996), Prader-Willi Syndrome (PWS, 2000), Small for Gestational Age (SGA) without adequate catch-up growth (2001), Idiopathic Short Stature (ISS, 2003), SHOX Deficiency (2006), and Noonan Syndrome (NS, 2007). In addition, rhGH therapy was FDA-approved for AIDS Wasting (1996), Adult GHD (1996), and Short Bowel Syndrome (2003) (**table**). Guidelines for the use of rhGH therapy have been reported previously by the American Association of Clinical Endocrinologists (AACE), Growth Hormone Research Society (GRS), and Pediatric Endocrine Society (PES, formerly The Lawson Wilkins Pediatric Endocrinology Society) (7-10).

Growth hormone registries have been an invaluable resource for determining the safety and efficacy of rhGH. These registries were originally mandated by the FDA in 1985 because rhGH was the first product approved in the U.S. derived from recombinant DNA technology. The FDA required Genentech to conduct a five-year phase IV post-marketing safety study. However, the National Cooperative Growth Study (NCGS) was extended as a phase IV post-marketing safety and efficacy study until 2010 (11). Other rhGH registries include GHMonitor^{SM} (Serono) and international registries American Norditropin® Studies: Web Enabled Research (ANSWER, Novo Nordisk), Genetics and Neuroendocrinology of Short Stature International Study (GeNeSIS, Eli Lilly), Pfizer International Growth Study (formerly Kabi International Growth Study, KIGS) and PATRO (Sandoz) as well as national and pharmaceutical registries in many individual countries. Some countries continued to mandate safety and efficacy registries for children receiving rhGH therapy. The cumulative safety and efficacy data from these multiple studies involve nearly 200,000 children treated for more than 600,000 patient-years. Numerous publications from these studies have educated the pediatric and adult endocrinology community about the safety and efficacy of rhGH therapy in children and adults.

Growth hormone registries have been and continue to be primarily conducted through entry of data relating to patient care without requiring adherence to specific practice guidelines. Thus, the data collected reflect the variation in clinical practice among the investigators who participate in the trial. Attempts have been made to use the registries to provide evidence to guide clinical practice (12) and to compare practice patterns to reported knowledge, attitudes and beliefs (13). However, one major weakness of these registries is the vast amount of data that are not entered by the participating sites. Although the study sites are compensated for the time required for data entry, due to the volume of data and work involved the number of fields required for payment were limited to high priority items. Therefore, items not required for payment are less likely to be recorded. However, the sheer volume of these registries overcomes these limitations to some degree.

This manuscript will focus on available safety data related to rhGH treatment of all conditions in children. Long-term safety will address effects that can occur after rhGH therapy is completed or in individuals who continue rhGH therapy into adulthood.

Safety

When considering a therapy for a medical condition, one of the guiding principles for physicians is Primum non nocere: First, do no harm. Therefore, when considering rhGH therapy for treatment of GHD and short stature, we need to consider the medical, physical, psychological and psychosocial impact of the disease or condition. The potential therapeutic benefit of rhGH therapy then needs to be balanced with the known and potential short- and long-term side effects. This chapter will define the short-term side effects as those that can occur while receiving rhGH therapy during childhood and the long-term side effects as those that occur after rhGH therapy has been discontinued or in those individuals who continue rhGH therapy into adulthood.

Short-Term Safety

Growth hormone registries have been an invaluable resource for determining the safety and efficacy of rhGH. Such large-scale post-marketing surveillance is necessary to accurately assess the safety profile of a drug due to the inability of clinical trials to identify rare side effects. In addition, spontaneous post-marketing adverse event reporting may be incomplete and does not allow for the determination of incidence rates for side effects. The cumulative safety data from the two largest studies (KIGS and NCGS) represent more than 120,000 children treated for nearly half a million patient-years. Based upon the most recent NCGS safety publication, the cumulative enrollment in the NCGS from December, 1985 to January 1, 2006, was 54,996 patients comprising 195,419 patient-years of treatment with Genentech's rhGH

Table. FDA Approved Indications for Growth Hormone Therapy by Manufacturer

	Genentech	Lilly	Novo Nordisk	Pfizer	Sandoz	Serono	Teva/Ferring
Indication							
Pediatric Growth Hormone Deficiency	**Protropin 10/85-12/02** Nutropin 3/94 Nutropin AQ 1/96 Nutropin Depot 12/99-6/04	Humatrope 3/87	Norditropin 3/97	Genotropin 8/95	Omnitrope 5/06	Saizen 10/96	Tev-Tropin 5/95 (Licensed to Ferring and renamed Zomacton in US in 5/15)
Chronic Renal Insufficiency	**Nutropin 11/93** Nutropin AQ 1/96						
Adult Growth Hormone Deficiency	**Nutropin 12/97** Nutropin AQ 12/97	**Humatrope 8/96**	Norditropin 3/97	Genotropin 10/97	Omnitrope 5/06	Saizen 11/04	Zomacton 1/18
Turner Syndrome	**Nutropin 12/96** Nutropin AQ 4/97	Humatrope 3/97	Norditropin 9/07	Genotropin 4/06			
Prader Willi Syndrome			Norditropin 2/18	**Genotropin 6/00**	Omnitrope 4/10		
Small for Gestational Age		Humatrope 3/09	Norditropin 10/08	**Genotropin 7/01**	Omnitrope 4/10		
Idiopathic Short Stature	Nutropin AQ 6/05	**Humatrope 7/03**	Norditropin 2/18	Genotropin 6/08	Omnitrope 8/1		
SHOX Deficiency		**Humatrope 11/06**					
Noonan Syndrome			**Norditropin 6/07**				
AIDS Wasting						**Serostim 8/96**	
Short Bowel Syndrome						**Zorbtive 12/03**	

***Bold** indicates that the product was the first to be approved for a given indication.*

Valtropin (LG Life, 4/07) and Accretropin (Cangene Corporation, 1/08) are rhGH products that have also been approved by the FDA, but are not yet commercially available in the U.S.

products (14). Additional safety data were collected with continued enrollment in NCGS until December, 2010 and data collection until June, 2011 when the study was discontinued. Based upon the most recent KIGS safety publication, the cumulative enrollment in KIGS from January, 1987 to August, 2008 was 58,603 patients comprising 197,173 patient-years of treatment with Kabi, Pharmacia and Pfizer's rhGH products (15). Additional safety and efficacy data continued to be collected until 2010 with the final safety report not yet published. The international ANSWER (5,797 subjects enrolled from January, 2002 through November, 2009) (16) and NordiNet IOS (initiated enrollment in 2006 with a goal of 17,000 subjects

over 10 years with migration of subjects from prior studies beginning in 2001) (17) registries continued to collect data through September, 2016 with the final safety report not yet published. The international GeNeSIS registry enrolled 22,311 from April 1999 to March 2010 comprising ~104,000 patient-years of treatment with Eli Lilly's rhGH products) (18,19). GHMonitor^{SM} enrolled 1335 subjects from January, 2003 through August, 2006 and is no longer collecting US data (20). Data for the international PATRO registry are not yet available.

The major safety questions that have been raised regarding rhGH therapy have been the risks of mortality and malignancy. The risk of mortality has focused particularly on sudden death in children with Prader-Willi Syndrome, aortic dissection in children with Turner Syndrome, and cancer survivors. The risk of malignancy has focused particularly on those children identified to be at increased risk for malignancy including cancer survivors and those with genetic conditions predisposed to tumor formation.

Short-Term Risk of Mortality

From the 54,996 patients enrolled in NCGS, there were 174 reported deaths, 19 (11%) of which were assessed as related to rhGH by the investigator, four were designated as not assessable, and no causality was provided for 21. The majority of the 19 deaths assessed as related to rhGH (12 of 19) were due to neoplasms (14). The other 7 deaths assessed as related to rhGH were isolated cases as follows: cerebral edema in a patient with a glioma resection, and cerebral edema in a patient with a mitochondrial myopathy and hypertrophic cardiomyopathy; arrhythmia in a patient with two renal transplants with hypertrophic cardiomyopathy and coronary atherosclerosis at autopsy; diabetic hyperosmolar coma in a patient with craniopharyngioma who was receiving rhGH at the time the hyperosmolar coma developed; depression after stopping rhGH in a 21-year-old male with thalassemia major, with subsequent death from an overdose of "street drugs"; complications of neurofibromatosis in a patient with an optic glioma, an odontoma, ataxia, and facial swelling; and sudden death in a 4-year-old male with PWS (14).

The GeNeSIS registry reported 42 deaths in 9504 rhGH-treated patients followed for ≥4 years (67,163 person-years of follow-up); the standardized mortality ratio (SMR) for the group was not elevated (SMR, 0.77; 95% CI, 0.56 to 1.05). SMR was significantly elevated in patients with history of malignant neoplasia (6.97; 95% CI, 3.81 to 11.69) and borderline elevated for those with other serious non-GH-deficient conditions (2.47; 95% CI, 0.99-5.09). SMRs were not elevated for children with history of benign tumors (1.44; 95% CI, 0.17 to 5.20), idiopathic GHD (0.11; 95% CI, 0.02 to 0.33), idiopathic short stature (0.20; 95% CI, 0.01 to 1.10), short stature associated with small for gestational age (SGA) birth (0.66; 95% CI, 0.08 to 2.37), Turner syndrome (0.51; 95% CI, 0.06 to 1.83), or short stature homeobox-containing (SHOX) gene deficiency (0.83; 95% CI, 0.02 to 4.65) (19).

Of the 14 children with history of malignant neoplasia who died, seven had a history of medulloblastoma, three had a history of astrocytoma, two had a history of leukemia, and one had a history of neuroblastoma; in addition, one had a history of Hodgkin lymphoma together with Down syndrome and CRI. Of the seven children who died with a history of medulloblastoma, four died after tumor recurrences, one died after developing acute myeloid leukemia as a second neoplasm, one died of complications of graft-vs-host disease after a bone marrow transplant for secondary myelodysplastic syndrome, and one died in a road accident. Death occurred after a second neoplasm in one patient with a primary diagnosis of acute lymphoblastic leukemia and one medulloblastoma survivor. A single death attributable to cerebrovascular disease (brainstem infarction) occurred in a child born SGA. However, the child had mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) as an underlying risk factor (19).

Mortality in Prader-Willi Syndrome

The annual mortality rate in PWS has been described as 3% across all age groups and is increased particularly in relation to complications of obesity (21). A review of all reported deaths in PWS conducted in 2007 identified 64 deaths in children up to age 19 years (42 male) (22). 28 (44%) of these children received rhGH treatment. Respiratory disorders were the most common cause of death (61%; 68% of rhGH-treated, 56% of untreated). 75% of deaths occurred in the first 9 months after the initiation of rhGH therapy. No significant differences were found between the groups in gender, prevalence of obesity or sleep apnea. More deaths in the untreated group occurred in children under the age of three (25 deaths, 22 untreated). However, deaths in those receiving rhGH were more evenly distributed by age.

It has been hypothesized that the causal connection between rhGH therapy and sudden death in PWS is the induction of tonsillar and adenoid hypertrophy. However, sleep studies have shown an improvement of sleep apnea, particularly central apnea and one death has been reported in a child with a normal polysomnograph before and after initiation of rhGH therapy (23,24). Recent deaths in individuals with PWS in trials of belanorib for treatment of hyperphagia and obesity have uncovered a potential increased risk of clotting events that could play a role in the death of children with this condition (25).

Increases in IGF-1 are associated with tonsillar hypertrophy. This would suggest that it may be safer to start at low doses of rhGH and titrate the rhGH dose using IGF-1 values. However, there are no studies to support this practice. In addition, children with PWS often have elevated IGF-1 levels in response to rhGH treatment, even at relatively low doses (26).

Tonsillectomy and adenoidectomy have been proposed as a treatment of sleep apnea. However, this treatment may be dangerous in children with PWS and may not improve the sleep apnea (23).

Although polysomnography has been recommended before starting rhGH treatment, particularly in the presence of snoring or obesity, children with a normal result still need close observation for sleep apnea and may benefit from repeat polysomnography during rhGH therapy. It should be emphasized that PWS is a condition with an inherently high mortality risk. Children with PWS, particularly with obesity, sleep apnea and respiratory illness, require careful clinical management whether or not they are receiving rhGH therapy. In those PWS children receiving rhGH therapy, awareness of the risk for sudden death should always be present, but particularly during the first 9 months of treatment. Although a causal link between rhGH therapy, respiratory illness and sudden death has not been established, discontinuation of rhGH therapy is recommended during respiratory illnesses. In addition, despite the potential metabolic and body composition benefits, rhGH therapy in severely obese children with PWS is contraindicated.

Mortality in Turner Syndrome

Aortic dissection, an often fatal event, occurs in 1–2% of the TS population and is usually preceded by dilatation of the aortic root and/or ascending aorta. Dissection occurs relatively early in life, at a median age of 35 years (range 18-61) (27). Longitudinal imaging every 5–10 yr to assess aortic diameters is recommended, even in those with a normal initial cardiac study (28). In the NCGS, five deaths from aortic dissection/rupture were reported among 5127 TS patients (14). The SMR in children with TS was not found to be elevated in the GeNeSIS registry (19).

Short-Term Risk of Malignancy

Since the inception of GH therapy, there has been ongoing concern about the potential for GH therapy to promote tumorigenesis either during therapy or in the period following therapy. Elevated levels of IGF-1 in the normal population have been reported to be associated with an increased risk of developing breast, prostate, and colon cancer in adults (29-31). In addition, GH and IGF-1 signaling have become adjunctive targets in the treatment of certain forms of cancer (32). However, a position paper from the 2015 GH safety workshop recognized that an evidence base supporting the value of IGF-1 monitoring for safety in children and indicating a safe upper limit for serum IGF-1 concentrations is lacking (33). Lessons from animal models have suggested that GH and IGF-1 play permissive rather than causative roles in tumorigenesis (34,35). However, since the first report of leukemia in a child receiving rhGH in 1988 (36), the possibility of an increased risk of cancer in patients receiving rhGH therapy has been investigated.

In the KIGS database, a total of 32 new malignant neoplasms were reported in 58,603 patients, versus the 25.3 expected (incidence, 16.4 per 100,000 patient-years; SIR 1.26; 95% confidence interval, 0.86-1.78) (15). These 32 new malignant neoplasms were diagnosed at a mean age of 11.9 years (range, 5.0-17.6 years). The mean duration of rhGH therapy in KIGS before the diagnosis of cancer was 3.6 years (range, 0.08-9.70 years). Eight of the 32 patients with a new cancer had been treated with rhGH before enrollment in KIGS for a median of 0.7 years (range, 0.06-4.5 years). In 6 of 7626 patients observed in KIGS after rhGH was discontinued, a malignant neoplasm was reported as long as 3.1 years after stopping rhGH. The mean dose of rhGH in patients in whom a neoplasm developed was 0.26 (+/-0.08) mg/kg/week (range, 0.12-0.48). Serum IGF-I levels measured 1 to 12 months before the onset of a neoplasm were only available for 7 patients. Three patients had IGF-I SDS >0 (range, 0.6-1.7), 4 patients had IGF SDS between –0.6 and –1.9, and none were above the normal range.

In the NCGS database, intracranial malignancies were reported in 243 patients, 44 of which were new-onset malignancies. Of the 44 new-onset intracranial malignancies, 15 had no predisposing factors. Extracranial malignancies, including leukemia, were reported in 87 patients with 63 new-onset malignancies. Of the 63 new-onset tumors, 21 had no predisposing factors (14). Using the 29 children with new-onset tumors with confirmed NCGS enrollment, a comparison to the risk of new-onset tumor in the general pediatric population yielded an SIR that was not significantly increased [1.12; 95% CI 0.75–1.61 (14)].

Based upon these results, the large volume of data from the KIGS and NCGS databases provide no evidence that rhGH therapy for childhood growth disorders results in an increased risk of developing cancer relative to that expected in the normal population (15). A literature review conducted by the PES Drug and Therapeutics Committee concluded: **1.** In children without known risk factors for malignancy, GH therapy can be safely administered without concerns about an increased risk for neoplasia. **2.** GH use in children with medical diagnoses predisposing them to development of malignancies should be critically analyzed on an individual basis, and if chosen, appropriate surveillance for malignancies should be undertaken. **3.** GH can be used to treat GH deficient childhood cancer survivors who are in remission with the understanding that GH therapy may possibly increase their risk for second neoplasms (37). The 2015 GH safety workshop found robust evidence for a lack of GH treatment effect on the development of a new primary cancer or a recurrent primary cancer. They reported that the evidence suggests that the risk of a second or subsequent neoplasm with GH treatment was present but diminished over time and that the association between GH

therapy and risk of second tumors is insufficient to preclude use of rhGH for licensed indications in children (33).

A recent meta-analysis of 16 observational studies comparing childhood cancer survivors (CCS) treated with rhGH to those not treated with rhGH showed that GH therapy was significantly associated with height gain (SDS: 0.61; 95% CI: 0.08 to 1.13) and not significantly associated with the occurrence of secondary tumors (OR: 1.10; 95% CI: 0.72 to 1.67) or tumor recurrence (OR: 0.57; 95% CI: 0.31 to 1.02). The same study analyzed 13 studies comparing CCS treated with rhGH to normal age- or sex-matched controls or controls with idiopathic GHD or short stature. In these studies, rhGH therapy was associated with either improved or unchanged risk of diabetes, abnormal lipid profiles and metabolic syndrome and was associated with improvements in quality of life (38).

Other Targeted Safety Events

Based upon data collected during preclinical trials and post-marketing surveillance registries, the product insert for the various rhGH products includes a number of safety-related issues. Many of these, including headaches and otitis media, are common in the general pediatric population and are minor concerns. Injection site lipoatrophy is a cosmetic concern that can be prevented by using appropriate rotation of injection sites. Injection site rashes and allergic reactions are concerns with any injectable material and may be related to the diluent rather than the rhGH. Borderline hypothyroidism (primary or secondary) may be uncovered with rhGH therapy. If hypothyroidism develops under these circumstances, it may be transient. Therefore, thyroid functions should be monitored during rhGH treatment. Fluid retention and edema are common side effects of rhGH therapy in adults, but are seen less in children and adolescents. Other targeted safety events including diabetes mellitus, intracranial hypertension, scoliosis, pancreatitis, adrenal insufficiency and slipped capital femoral epiphysis are described below. In addition, a section describing concerns about rhGH therapy for short stature due to chronic renal insufficiency prior to or following renal transplantation is included.

Diabetes Mellitus

Diabetes Mellitus (DM) has been reported in 65 patients; 37 Type 1 DM (T1DM), 20 Type 2 DM (T2DM) and 8 unclassifiable (14). The 33 T1DM cases, compared with that expected and adapted to an age-matched population yielded a SIR of 0.90 (observed 33; expected 36.8). The mechanism of rhGH action would make an increase in the incidence of T1DM unexpected. However, due to the relative insulin resistance caused by increased GH secretion or rhGH administration, it would be more likely for a child to develop T2DM while receiving rhGH therapy. If the 8 unclassifiable cases are considered T2DM, the incidence would be 14 per 100,000 patient years. However, it is difficult to compare this incidence to the general pediatric population due to the changing incidence of T2DM over the years of the NCGS database. It is also difficult to determine if rhGH therapy causes a long-term increase in the risk of T2DM since the insulin resistance would be reduced once rhGH therapy was discontinued. In adults treated with rhGH as children for growth failure associated with SGA, insulin resistance has been shown to revert to normal following cessation of rhGH therapy and was comparable to control individuals who were SGA but had spontaneous catch up growth (39).

Intracranial Hypertension

Although intracranial hypertension (IH), also known as pseudotumor cerebri, has been identified in children receiving rhGH therapy, no known mechanism has been identified. The annual incidence of IH in the general pediatric population is 0.9 per 100,000 (40). IH was reported in 61 confirmed NCGS patients with the majority of events occurring within 6 months of starting rhGH(14). Thus the incidence of IH in children receiving rhGH therapy reported in the NCGS is 31 per 100,000 patient years (14). IH was reported in 41 confirmed KIGS children receiving rhGH therapy with an incidence of 28 per 100,000 patient years (41). The highest incidence is seen in children with TS, organic GHD, PWS, and CRI (14,41). Even though only 2 cases of IH have been seen in ISS, this is still higher than the general population (14). It is unclear whether this is an ascertainment bias. However, IH in children receiving rhGH is generally resolved with discontinuation of rhGH therapy for a brief period followed by resumption of rhGH at a lower dose.

Scoliosis

rhGH therapy does not cause scoliosis. However, it can make it worse so that previously unrecognized scoliosis becomes evident or known scoliosis progresses. Scoliosis was reported in 238 NCGS patients of which 76 were preexisting. Scoliosis was more common in TS and PWS. Among the 64 patients with serious scoliosis, 37 reported surgical treatment. It is recommended that clinicians monitor regularly for the development or worsening of scoliosis. If scoliosis is present and/or worsening, involvement of a pediatric orthopedist is recommended. rhGH therapy in children with severe or worsening scoliosis should be monitored closely and should involve ongoing communication between the pediatric orthopedist and pediatric endocrinologist.

Pancreatitis

Pancreatitis has been reported in 10 children receiving rhGH therapy in the NCGS database and one individual 5 months after discontinuation of rhGH therapy. These data provide an incidence of 5.2 per 100,000 in rhGH treated individuals, yielding an SIR of 1.44 and 95% CI of 0.70–2.67(14). The annual background incidence of pancreatitis is 3.6 per 100,000 in

the pediatric age group (42). These data fail to demonstrate a clearly significant relationship between rhGH therapy and pancreatitis.

Adrenal Insufficiency

Concern about adrenal insufficiency (AI) in children with multiple pituitary hormone deficiencies has arisen due to the impact of GH on cortisol metabolism. The microsomal enzyme 11ß-hydroxysteroid dehydrogenase type 1 (11ßHSD-1) is required for conversion of cortisone to its active metabolite, cortisol. GH inhibits 11ßHSD-1 activity (43). Therefore, children with unrecognized central AI may develop an adrenal crisis due to rhGH therapy. Alternatively, individuals with known central AI may require higher maintenance and stress doses of steroids when rhGH therapy is initiated (44). The need for close dose monitoring and possible increase would be particularly true in individuals receiving prednisone or cortisone acetate that require 11ßHSD-1 activity for conversion to cortisol. In patients with organic GHD (OGHD) and idiopathic panhypopituitarism in the NCGS, 11 events consistent with acute AI, including four deaths, were recorded (14). The time from onset of rhGH therapy to the event of AI ranged from within 24 hours of initiation to 7.4 years, with a mean of 2.5 years. Five nonfatal cases were assessed as serious by the investigator. Although rhGH may facilitate the occurrence of AI, patients with hypopituitarism are at lifelong risk for AI and sudden death whether or not they are receiving rhGH (45,46).

Slipped Capital Femoral Epiphysis

The annual incidence of slipped capital femoral epiphysis (SCFE) in the general pediatric population is 2-13 per 100,000 (47,48). The risk of SCFE is increased in obesity, hypogonadism, multiple pituitary hormone deficiency and rapid growth during puberty. SCFE was reported in 93 confirmed NCGS patients receiving rhGH(14). Thus the incidence of SCFE in children receiving rhGH therapy reported in the NCGS is 48 per 100,000 patient-years (14). SCFE was reported in 52 confirmed KIGS children receiving rhGH therapy with an incidence of 73 per 100,000 patient-years (41).The highest incidence is seen in children with TS, organic GHD, and CRI (14,41). Wider growth plates and relative osteopenia seen in GHD, SHOX deficiency in TS, and metabolic bone disease in CRI may predispose the growth plates to slip during rapid growth (14,41).

Chronic Renal Insufficiency Post-Transplant

Primarily, there has been concern that rhGH therapy would increase the frequency of solid organ rejection. Data from the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) showed no increase in rejection episodes in children receiving rhGH therapy after renal transplant compared to children not receiving rhGH. A potential increased risk of rejection was seen in patients receiving rhGH with a history of frequent previous rejection episodes (49,50). However, the risk of rejection is also increased in children with a history of frequent previous rejection episodes who have not received rhGH. The use of rhGH pre-transplant was associated with a borderline higher risk for post-transplant lymphoproliferative disorder (PTLD, odds ratio 1.88, 95% CI = 1.00–3.55, p = 0.05). In contrast, use of rhGH during dialysis or post-transplant only was not associated with a higher risk for PTLD (51). Because of these concerns, rhGH therapy in children following renal transplantation should be carefully considered by a multi-specialty team including pediatric nephrologists, pediatric endocrinologists and transplant surgeons.

Long-Term Safety

For the purposes of this chapter, long-term side effects are defined as those that occur after rhGH therapy has been discontinued or in those individuals who continue rhGH therapy into adulthood. In children with organic GHD and severe isolated GHD, rhGH therapy should be considered replacement therapy and if replacement doses are not excessive would not be expected to have long-term side effects. However, subcutaneous doses given once daily are unlikely to match the natural profile of pulsatile GH secretion. If larger doses are given for OGHD or IGHD, or when treating conditions without GHD, then there may be more risk of long-term negative effects. The relative safety of rhGH during therapy, as documented in numerous publications, and described earlier in this manuscript makes long-term side effects less likely.

Measurement of IGF-1 and IGFBP-3 levels during therapy started as a measure of efficacy and compliance (52). With the identification of a possible correlation between high IGF-1 levels and an increased risk of cancer in adults, monitoring IGF-1 and IGFBP-3 has become a safety practice (52). However, it is unclear whether elevated levels of IGF-1 or IGFBP-3 during childhood correlate with any adverse effects in adulthood (33). Studies showing an increased risk of cancer with high IGF-1 levels in adults actually show that the highest risk occurs in those with elevated IGF-1 and low IGFBP-3 (52). This may be due to elevated levels of free IGF-1, reduced IGF-independent actions of IGFBP-3 or both. Low levels of IGF-1 with high IGFBP-3 in adulthood are associated with an increased risk of cardiovascular disease (52).

Children treated with rhGH typically have low or low normal levels of IGF-1 prior to therapy. During rhGH therapy, IGF-1 and IGFBP-3 levels increase in tandem, a combination with a theoretical low cancer risk profile. The degree of elevation of IGF-1 and IGFBP-3 during rhGH therapy is dependent upon the rhGH dose, compliance and GH-responsiveness. Following completion of rhGH therapy, IGF-1 and IGFBP-3 levels are likely to revert to pre-treatment ranges with low IGF-1 and IGFBP-3 that have a low cancer risk profile. It is unclear

whether the temporary, though prolonged, elevation of IGF-1 and IGFBP-3 levels during rhGH therapy has any long-term impact on the cancer risk or any other health risk factors. Although it has been proposed as a safety measure, it also remains unclear whether monitoring IGF-1 and IGFBP-3 levels, targeting IGF-1 and/or IGFBP-3 values to a certain range, or reducing rhGH doses if IGF-1 levels are high, has any impact on long-term safety of rhGH therapy (53,54).

Although GH registries have been invaluable in the determination of safety and efficacy during rhGH therapy, once children discontinue therapy, they are not typically entered into the registries and frequently no longer see the pediatric endocrinologist. Therefore, long-term safety data in this population are sparse. There have been several studies that have attempted to investigate long-term effects of rhGH therapy in childhood. In one study, the risk of cancer was investigated in adults who had received pituitary-derived growth hormone between 1959 and 1985 (55). There was no overall increase in the incidence of cancer in this study. However, this study raised concerns about an increased risk of colorectal cancer (SIR 7.9; 1.0-28.7, p=0.05) and Hodgkin's lymphoma (SIR 2.3; 0.3-8.5, p=0.42) occurring in individuals with IGHD. Two cases of each type of these cancers were identified in this population. One of the cases of colorectal cancer occurred in an individual with an unclear history of possible polyposis coli. If that case is excluded, neither tumor had a significantly increased incidence compared to the normal population.

In the Childhood Cancer Survivor Study, long-term follow-up of childhood cancer survivors has shown that there is an increased risk of second neoplasm in GH treated children that develop years after treatment (56). In this study, the rate ratio of GH-treated survivors developing a second neoplasm was 2.15 (95% confidence interval, 1.3–3.5; P <0.002) compared with non-GH-treated survivors (56).

This study included data from children who received pituitary-derived GH, rhGH or both. Meningiomas were the most common second neoplasm (9/20). All individuals who developed meningiomas as second neoplasms had previously received radiation to the head or brain (56). After adjusting for covariates, the relative risk of death for GH-treated patients compared with those not treated with GH was not statistically increased (1.20; 95% CI, 0.81–1.79; P = 0.36).

Because of the unanswered questions regarding long-term safety of rhGH therapy, further long-term follow-up studies are necessary. These studies could be prospective cohort studies or cross-sectional studies. One such effort is the retrospective observational study entitled Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE). On December 10, 2010, the French drug agency Agence Francaise de Securite Sanitaire des Produits de Santé and the European Medicines Agency (EMA) issued press releases based upon preliminary mortality data from the French portion of this study, Santé Adulte GH Enfant (SAGhE) regarding potential increased mortality in adults previously treated with rhGH (57,58). According to the reports, the French SAGhE study evaluated data from about 7,000 patients who had received rhGH during childhood, initiated between 1985 and 1996, for treatment of IGHD (~75% of the cohort) or ISS/SGA. They reported an increased risk of all cause mortality (93 deaths) compared to the general French population (70 deaths estimated), with an abnormally high death rate due to cerebrovascular complications and bone tumors. The risk was also reported to be associated with higher doses of rhGH.

The Committee for Medicinal Products (CHMP) for Human Use of EMA reviewed the available data and determined "that there is no need for regulatory action based on the presented data." On December 16, 2010, the CHMP of EMA issued a following statement reporting their plans for further review of the safety of rhGH and stated: "While this review is ongoing, the CHMP confirms that there is no immediate concern. However, prescribers are reminded to strictly follow the indications and the approved doses. The maximum recommended dose of 50 mcg/kg weight/day for somatropin-containing medicines should not be exceeded" (59). The FDA issued a similar report on December 22, 2010 (60). The EMA and FDA reported that they would review all available information on the potential risk of rhGH therapy and communicate any new recommendations once the review was complete. The FDA issued the following statement: "At this time, FDA believes the benefits of recombinant growth hormone continue to outweigh its potential risks" (60).

A subsequent peer-reviewed report from the French participants in the SAGhE study demonstrated higher all-cause mortality rates in individuals treated with GH during childhood for idiopathic GHD and non-GHD populations compared with the French general population (61). In contrast to the French cohort, the SAGhE data from Sweden, Belgium, and the Netherlands failed to demonstrate any difference in death rates between GH-treated and the normal population (62).

Subsequent analysis of the cerebrovascular complications in the French cohort reported 5 subarachnoid hemorrhages, 3 intracerebral hemorrhages and 3 ischemic strokes. When compared to Dijon and Oxford population-based registries as reference data sets, there was a higher risk of hemorrhagic stroke (standardized incidence ratio, SIR 3.5 to 7.0) and particularly subarachnoid hemorrhage (SIR 5.7 to 9.3). As a result of these findings, the authors recommended that patients treated with GH be advised about this association and called for further studies to evaluate the potentially causal role of GH treatment in these findings (63). It is noteworthy that three hemorrhagic strokes were reported in GeNeSIS, all in individuals with known risk factors.

Small size at birth has been associated with increased mortality. Using a conventional model which adjusts for age, gender and calendar-year, the ratio of observed/expected deaths was increased (21/14.68) in childhood rhGH-treated IGHD, ISS, and SGA patients compared with the general Swedish population. However, when applying an advanced sex-specific mortality model adjusting for birth characteristics, the ratio of observed/expected deaths (21/21.99) was not increased (64). These data emphasize the need to incorporate patient characteristics, including birth data, and appropriate reference populations into analysis of long-term risk of GH therapy.

A preliminary analysis of the French SAGhE cohort was performed to evaluate the risk of Type 1,2 or gestational diabetes mellitus in young adults who received rhGH treatment during childhood. When compared to the general French population, no increase in the risk of diabetes mellitus was identified (65).

The preliminary report of the French cohort did not find any increase in cancer-related mortality. However, there was an increase in mortality related to bone tumors, with a standardized mortality ratio of 5.00 [3 observed vs. 0.6 expected, confidence interval (CI) 1.01–14.63] (61,66). In contrast, the SAGhE data from Sweden, Belgium, and the Netherlands failed to demonstrate any difference in death rates from cancer between GH-treated subjects and the normal population (62,67).

Analysis of cancer risk and mortality in the whole SAGhE cohort (Belgium, France, Germany, Italy, the Netherlands, Sweden, Switzerland, and United Kingdom) was performed by comparing the incidence rates in individuals who received rhGH as children to country-specific cancer incidence and mortality data (68). In individuals who received rhGH for treatment of isolated growth failure due to isolated GHD, ISS or prenatal growth failure, there was no increased site-specific or overall cancer risk. In contrast to preliminary data from the French SAGhE cohort suggesting increased mortality related to higher doses of rhGH, the risk of cancer incidence decreased with increasing mean rhGH dose and cumulative dose. The risk of cancer mortality was not related to mean rhGH dose but was lower with cumulative rhGH dose.

In individuals without isolated growth failure or cancer, the risk of overall cancer incidence (SIR 1.4, 95% CI, 1.1 to 1.9) and mortality (SMR = 2.2; 95% CI 1.3 to 3.7), and of bone (SIR = 4.1; 95% CI, 1.3 to 12.6) and bladder (SIR = 27.8; 95% CI, 7.0 to 111.3) cancer incidence was significantly elevated based upon a small number of cases.

In individuals with a cancer diagnosis prior to rhGH therapy, there was an increase in cancer risk and mortality overall and with multiple cancer sites. The risk of mortality decreased with time elapsed since starting rhGH treatment and duration of treatment. Cumulative rhGH dose did not significantly affect mortality or incidence. However, mortality but not incidence increased with increasing mean rhGH dose. It remains difficult to determine if rhGH therapy causes this change in cancer risk or mortality or if this change is due to other underlying characteristics of the cancer survivors.

Interpretation of SAGhE results will require consideration of country-specific issues such as methods of cohort assembly, follow-up, death reporting mechanisms, and reference population utilized. The preliminary overall mortality results and completed cancer risk and cancer mortality results of SAGhE regarding potential mortality related to rhGH therapy emphasize the need for ongoing long-term studies of adults previously treated with rhGH. It is also necessary to ensure that appropriate reference or control populations are evaluated to determine if the risk is related to rhGH therapy or a predisposition related to the underlying condition (IGHD, ISS or SGA). It is unclear whether there is a selection bias for individuals participating in such a study that may influence the risk of reporting adverse events. It is noteworthy that studies of cancer survivors have shown the increased risk of second neoplasms compared to the reference population was reduced simply by extension of the follow-up period (56). This argues that long-term studies are necessary to determine the risk of long-term side effects related to rhGH therapy. Similar studies should be considered in the U.S. where the prescribed rhGH doses are typically higher than in Europe. However, tracking individuals previously treated with rhGH over time may be difficult.

Overall, the rhGH short-term safety profile is reassuring with the fewest adverse events occurring in children with ISS. It is unclear whether rhGH treatment during childhood has any long-term impact on morbidity and mortality. However, since short stature has low morbidity and mortality, it would not be acceptable to expose children to even a small increase in the long-term risk of a serious adverse event related to rhGH treatment for the relative improvement in growth. Therefore, the pediatric endocrinology community, rhGH manufacturers, research funding and regulatory agencies need to work together to design studies that will identify the long-term risk of rhGH therapy (69).

Future Directions

This manuscript has focused on currently available data regarding safety of rhGH therapy. Long-acting rhGH (LAGH) was previously available as Nutropin Depot. The safety profile of this medication was similar to daily rhGH (70). However, the medication was only commercially available for 4.5 years and no long-term studies have been reported. Several forms of long-acting rhGH are currently in development and may soon be commercially available (69,71,72). The currently

available safety data from clinical trials of LAGH in children and adults have not shown any evidence of new side effects from this class of medications. However, routine clinical trial monitoring is unlikely to uncover more suitable metabolic differences that may occur when transitioning from daily rhGH injection to longer injection intervals (weekly or greater). Daily rhGH given at bedtime provides peak GH levels overnight and may not impact the daytime metabolic actions of GH. However, persistent GH as a result of treatment with LAGH products may have differing metabolic impacts. In addition, side effects related to each unique LAGH molecule will need to be monitored including neutralizing anti-GH antibodies. If these long-acting rhGH preparations are approved, it will be important to establish their short-and long-term safety and efficacy. In light of the SAGhE study raising potential new concerns for long-term health effects in children with growth disorders, further long-term studies are needed to better understand the potential impact of underlying individual characteristics and treatment exposures on the long-term health and well-being of individuals who have received rhGH and/or LAGH therapy in childhood. Appropriate control populations will be crucial to interpret data from these long-term studies. Due to the relatively small numbers of individuals receiving rhGH and/or LAGH therapy and the power needed to accurately identify rare associations, a lifelong global registry of individuals previously treated with GH products is needed. Such a registry could be developed as a cloud-based data repository involving patient, parent and provider-reported data including patient characteristics, concomitant medications, rhGH or LAGH dose exposure, psychosocial outcomes (i.e. salary, relationship status, quality of life) and adverse events. Efforts to link individuals in a registry with their data from previous clinical trials and registries would be beneficial to expand data available. Linkage of data across registries is also important due to insurance-mandated rhGH or LAGH brand changes. A lifelong global cloud-based registry of individuals treated with rhGH or LAGH under the supervision of an independent safety monitoring/epidemiology research group should be supported by the multiple bodies funding research in childhood growth disorders and long-term health outcomes including government agencies, philanthropic organizations, and the pharmaceutical industry (69).

Disclosure

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Long-Acting Growth Hormone Preparations in the Treatment of Children

Rayhan A Lal[1,2], MD, Andrew R. Hoffman[2,3], MD

[1]Division of Endocrinology, Department of Pediatrics, Stanford University School of Medicine, Stanford, California, USA
[2]Division of Endocrinology, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA
[3]Medical Service, VA Palo Alto Health Care System, Palo Alto, California, USA

Corresponding author: Rayhan Lal, MD, Stanford University School of Medicine, Room G-313 Medical Center, 300 Pasteur Drive, Stanford, CA 94305, USA, Tel: 650-498-7351, Fax: 650-725-8375, E-mail: inforay@stanford.edu

Abstract

Human growth hormone (hGH), which had been in use since 1958, was supplanted by recombinant human growth hormone (rhGH) in 1985 for those with growth hormone deficiency (GHD). Adherence to daily subcutaneous growth hormone is challenging for patients. Thus, several companies have pursued the creation of long acting rhGH. These agents can be divided broadly into depot formulations, PEGylated formulations, pro-drug formulations, non-covalent albumin binding GH and GH fusion proteins. Nutropin Depot is the only long acting rhGH ever approved by the U.S. Food and Drug Administration, and it was removed from the market in 2004. Of the approximately seventeen candidate drugs, only a handful remain under active clinical investigation or are commercially available.

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Background

In 1958, Raben reported the clinical use of human growth hormone that had been purified using glacial acetic acid from pituitary glands harvested at autopsy. By injecting this purified pituitary extract two-three times each week, he was able to achieve increased growth in a teenager with hypopituitarism (1). Subsequently, the National Pituitary Agency was formed to supervise the collection of human pituitary glands, to arrange for the extraction and purification of the human growth hormone (hGH) from these glands, and to distribute this precious, scarce hormone to pediatric endocrinologists for the treatment of children with GH deficiency (GHD). While early preparations of this cadaveric-derived hGH contained GH polymers and other contaminating materials, the methodology for purification improved with increasing complexity over time (2). Pharmaceutical companies also entered this market, and ultimately, more than 30,000 people received pituitary-derived hGH.

In 1985, a 20-year-old man who was followed at the Stanford Endocrine Clinic for hypopituitarism and diabetes and who had received cadaveric hGH since the age of 3, died of Creutzfeld-

Jakob disease. The NIH was quickly notified, and several other cases were soon identified, leading to the withdrawal of pituitary-derived hGH from use in the United States (3). Fortunately, a recombinant human GH (rhGH) with an added methionine was approved by the FDA and marketed by Genentech (South San Francisco, USA) in the same year, and subsequently, several companies introduced native rhGH to the market for use in both children and adults with GHD.

With the availability of unlimited amounts of rhGH, clinicians began to prescribe rhGH as a daily subcutaneous injection. Adherence to a daily injection has not been optimal, and it has been postulated that by facilitating increased adherence, a long-acting GH formulation that could be given weekly, biweekly or even more infrequently might lead to better growth in children (4). Studies in which rhGH was given as a constant subcutaneous infusion for as long as 6 months showed that IGF-I levels could be maintained, suggesting that desensitization of the GH receptor did not occur when it was exposed to constant GH levels (5). A number of pharmaceutical companies began to develop long-acting GH preparations. We will discuss a representative group of these novel GH formulations that have been tested in humans (6).

Depot Formulations

The first entry in the long acting rhGH field was Nutropin Depot, a product made by Genentech (South San Francisco, USA) and Alkermes (Dublin, Ireland), which consisted of rhGH that was encapsulated in microspheres composed of a poly(lactide coglycolide) copolymer that was fully biodegradable. In a study of 74 prepubertal children with GHD, monthly or twice monthly administration of Nutropin Depot led to annualized growth rates of 8.4 cm/year after 6 months of therapy, and this drug was approved by the FDA for use in children with GHD (7). However, it was necessary to deliver large volumes of this viscous medication, and often more than one injection at a time was needed to deliver the proper dose to children. The injections required a large bore needle, and a large lump was apparent for several days after the injection. In a subsequent study of adults with GHD, Nutropin Depot was shown to be effective in decreasing truncal and visceral adipose tissue (8). The drug was never approved for adults, and it was removed from the market in 2004.

Declage or Somatotropin Biopartners (LB03002) is produced by BioPartners (Los Angeles, USA) in conjunction with LG Life Sciences (Seoul, South Korea). It is an rhGH incorporated into sodium hyaluronate which is suspended in an oil base of medium-chain triglycerides (MCT) before injection. Hyaluronidase present in tissue degrades the microspheres, releasing rhGH. The pharmacokinetics and pharmacodynamics were first evaluated by Bidlingmaier and colleagues in 2006 among 6 men and 3 women with adult GHD on a stable daily rhGH regimen. Participants underwent a 4-week washout followed by weekly LB03002 administration for five weeks. Maximal serum GH concentration doubled, dose-normalized area under curve was not significantly different, mean maximal serum IGF-1 was 34-41% greater and normalized-to-rhGH-dose IGF-1 area under curve was comparable (9). In 2009 a randomized, comparator-controlled, assessor masked phase 2 study was performed on 37 pre-pubertal, rhGH-naïve children. They received rhGH 0.03mg/kg for 7 days, followed by a 3-week washout then one of three different doses of LB03002 weekly for 3 months. Maximal serum GH concentrations increased up to 4-fold, GH area under curve remained stable over 3 months, maximal IGF-1 progressively increased with normal IGF-1 standard deviation scores achieved after 3 months and normal IGFBP-3 scores after 1 week treatment (10). A 2011 placebo-controlled clinical trial of LB03002 for 26 weeks in 152 adults with GHD demonstrated significantly increased IGF-1 levels and reduced mean fat mass (11). These changes were sustained in a 1-year follow-up study (12). A prospective sub-study to the year-long phase III trial also showed reduction in fat mass with reduced leptin and increased ghrelin, but no change in glucose or lipid metabolism (13). In 2012, Péter and colleagues confirmed the efficacy and safety of LB03002 at doses 0.5-0.7 mg/kg/wk versus daily rhGH in a 3-year trial for GH-naïve prepubertal children with GHD (14). A 1-year pediatric trial in Korea compared weekly LB03002 to daily rhGH and demonstrated comparable mean height velocity, which was also shown in the 2 year phase III multinational trial (15-16). While LB03002 was approved in Europe, it has not been marketed there. It is available for use in children in South Korea.

PEGylated Formulations

The addition of polyethylene glycol (PEG) moieties to a protein has been shown to extend its half-life and even decrease its antigenicity. PEGylated drugs are currently being marketed for the treatment of numerous diseases, including anemia (Amgen PEGylated erythopoeitin) and neutropenia (Amgen PEGylated G-CSF). A PEGylated GH antagonist manufactured by Pfizer (New York City, USA), pegvisomant, is an effective therapy for acromegaly. Ambrx (La Jolla, USA) developed ARX201, a pegylated GH agonist that was effective in increasing serum IGF-I levels in patients with GHD (17). However, preclinical studies in primates showed at autopsy that the PEG was accumulating in the ependymal cells of the choroid plexus. While it is not clear whether this pathologic finding had any clinical significance, Ambrx stopped developing this drug. Both Novo Nordisk (Bagsværd, Denmark) and Pfizer (New York City, USA) also ended their programs of PEGylated GH molecules (NNCI126-0083 and PHA-794428, respectively).

One PEGylated formulation is currently in use for the treatment of GHD in children in China. GeneScience Pharmaceuticals' (Changchun, China) Jintrolong is a PEGylated long-acting rhGH designed to be injected weekly. This drug was first tested in 2015 during a phase 1 trial on 12 children with GHD. Participants received rhGH 0.0286 mg/kg daily for 7 days, followed by 4 week washout and 6 weeks of Jintrolong 0.2mg/kg weekly for 6 weeks. Elimination rate was slower than daily rhGH without significant accumulation and serum IGF-1 was comparable between treatment regimens (18). The phase 2 and 3 trials were conducted in 6 hospitals in China and published in 2017. Participants were treatment-naïve children with GHD; 108 were enrolled in the phase 2 trial and 343 in the phase 3 trial. The phase 2 study demonstrated the safety and efficacy of Jintrolong at 0.2mg/kg weekly for 25 weeks versus 0.1mg/kg weekly dosing and daily rhGH. The 25 week phase 3 study revealed greater height velocity increase and standard deviation height scores for weekly Jintrolong versus daily rhGH (19).

Pro-Drug Formulations

Ascendis Pharma (Hellerup, Denmark) TransCon sustained-release rhGH is an inactive prodrug consisting of rhGH transiently bound via proprietary linker to an inert carrier, methoxypolyethylene glycol (mPEG). The linker undergoes controlled autohydrolysis dependent on pH and temperature. A phase 1 randomized trial among 44 healthy male subjects compared 4 different doses of weekly TransCon rhGH prodrug to 2 different doses of daily rhGH. They found no adverse events, injection site reaction differences and no binding antibody formation. Measures of GH and IGF-1 were comparable at similar doses (20). Follow-up phase 2 trials among 37 adults with GHD and 53 GH-naïve prepubertal children with GHD revealed similar safety and efficacy of TransCon weekly rhGH and daily rhGH (21-22). At the time of this writing, phase 3 testing is underway.

Non-Covalent Albumin Binding GH

Novo Nordisk's (Bagsværd, Denmark) Somapacitan (NNC0195-0092) is rhGH with a single point mutation to which a terminal fatty acid is attached with noncovalent albumin-binding properties. The albumin binding is predicted to increase half-life through reduced clearance (23). This is the same technology that is used in their long-acting insulin detemir. A 2014 randomized, placebo-controlled single center trial of 105 healthy male subjects was performed in Germany. The drug was well tolerated without immunogenicity concerns at the 4 doses tested and demonstrated a dose-dependent increase in measured IGF-1 (24). A phase 1 trial among 34 adult men with GHD also demonstrated tolerability and comparable efficacy to daily rhGH based on serum IGF-1 (25). Similar findings were reported in the phase 1 trial of 32 prepubertal children with GHD (26). A 26 week randomized, controlled phase 3 trial performed in 6 countries was recently published. 92 participants with adult GHD were treated with weekly Somapacitan or daily rhGH administered subcutaneously by pen. Participants felt dosing was more convenient, IGF-1 standard deviation scores were maintained within a therapeutic target, and the medication was well tolerated (27).

GH Fusion Proteins

The Korean biotechnology firm, Genexine (Seongnam, South Korea) in collaboration with Handok (Seoul, South Korea) has produced HyTropin (GX-H9), which is rhGH fused to Genexine's proprietary hybrid Fc (hyFc) platform. The hyFc is created from IgD, which provides the greatest hinge flexibility and low antibody-dependent cellular cytotoxicity, as well as IgG4 which binds to the protective neonatal Fc receptor and exhibits low complement-dependent cytotoxicity (28). Phase 2 trials with 7-14 day administration in adult and children have demonstrated acceptable tolerability without significant immunogenicity and comparable biochemical efficacy. The company is seeking FDA approval to begin phase 3 trials.

Teva Pharmaceutical Industries (Petah Tikva, Israel) studied a long acting GH analog (TV-1106) that fused rhGH to human serum albumin, a molecule that had been developed by Human Genome Sciences as albutropin (29). While this drug initially showed promise as an effective treatment for childhood GHD, the development of potentially blocking or inactivating antibodies led the company to abandon the drug.

MOD-4023 is a chimeric product generated by fusing three copies of the 28 carboxy-terminal residues of human chorionic gonadotropin (hCG) beta subunit to the coding sequence of hGH developed by OPKO Health (Miami, USA) and Pfizer (New York City, USA) (30). The phase 2 study in 54 GH-deficient adults with once weekly dosing demonstrated favorable safety and efficacy with IGF-1 values comparable to daily rhGH dosing (31), but the phase 3 trial did not show noninferiority to daily rhGH, and there are no ongoing studies in adults. Similarly, a multicenter, open-label, randomized, controlled phase 2 study among 53 prepubertal children with GHD receiving weekly MOD-4023 for one year revealed promising safety and tolerability with 6 month annualized height velocity above 12 cm/year (32). Subsequent pharmacokinetic and pharmacodynamic studies in children established that sampling 4 days following dose administration allowed best estimation of mean IGF-1 SDS (33). Studies are continuing for the treatment of GHD in children.

In order to extend the half-life of the GH molecule, Versartis developed Somavaratan or VRS-317, by adding long unstructured hydrophilic strings of amino acids were to the ends of the GH molecule. While this GH analog was able to increase height velocity in children with GHD when given weekly or even less frequently, a phase 3 study showed that it was inferior to daily rhGH in its ability to increase height, and therefore this product was also discontinued (34).

Conclusions/Future Directions

Many long-acting rhGH formulations have been designed and tested in children and adults with GHD, and while no product is currently available for use in the United States, a PEGylated GH is being used in China and a depot formulation is available in South Korea (**table 1**). Over the past few years, a number of long-acting rhGH molecules have been discontinued, either because they are less effective than daily rhGH or because

Table 1. Summary of long-acting rhGH preparations

Class	Company	Product	Design	Status
Depot Formulations				
	Genentech / Alkermes	Nutropin Depot	Encapsulated in Polylactide-Coglycolide Microspheres	FDA Approved. Removed from market in 2004.
	BioPartners / LG Life Sciences	Declage LB03002	rhGH incorporated into sodum hyaluronate and suspended in MCT for injection	Available for use by children in South Korea. Approved in Europe but not marketed.
PEGylated Formulations				
	Ambrx	ARX201	30-kDa PEG incorporated into rhGH	Drug development stopped due to PEG accumulation in ependymal cells of choroid plexus.
	Novo Nordisk	NNCI126-0083	43-kDa PEG attached to rhGH	Drug development stopped.
	Pfizer	PHA-794428	Branched 40-kDa PEG attached to amino end of GH	Drug development stopped.
	GeneScience Pharmaceuticals	Jintrolong	40-kDa PEG attached to rhGH	Available for use by children in China.
Pro-drug Formulations				
	Ascendis Pharma	TransCon Growth Hormone	rhGH transiently bound to mPEG	In phase 3 testing.
Non-Covalent Albumin Binding GH				
	Novo Nordisk	Somapacitan NNC0195-0092	rhGH with a single point mutation attached to a terminal fatty acid	In phase 3 testing.
GH Fusion Proteins				
	Genexine / Handok	HyTropin GX-H9	rhGH fused to IgD and IgG4	Pending phase 3 testing.
	Teva	TV-1106	rhGH fused to albumin	Blocking/inactivating antibodies developed, drug abandoned.
	OPKO Health / Pfizer	MOD-4023	rhGH fused to 3 copies of carboxy-terminal of hCG's beta subunit	Phase 3 studies in adults completed at end of 2016, phase 3 testing in children underway.
	Versartis	Somavaratan VRS-317	rhGH fused to hydrophilic strings of amino acids	Discontinued due to inferiority to daily rhGH in increasing height in children

of potential side effects or complications (35). While there is ample data testifying to the relative safety of daily rhGH therapy in children (36), it will be necessary for the companies that market the long-acting rhGH preparations and for the physicians who prescribe them to perform thorough post-marketing surveillance to determine the safety of these novel molecules (37). It will be vital to learn whether constant exposure to GH action leads to acromegaly in the long run. Moreover, it is possible that the modifications introduced on some of these preparations could cause new side effects that we have not seen with daily rhGH. Finally, it would be of great interest to learn if giving a long-acting rhGH preparation leads to better adherence and better growth.

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Disclosure

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Dr. Hoffman has consulted for Ascendis, GeneScience, Genexine, NovoNordisk, Pfizer and Versartis

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SI and Metric Units for Plasma, Serum, or Blood

	Metric unit	Conversion factor	SI unit
Acetoacetate	mg/dl	97.95	µmol/l
Acetone	mg/dl	172.2	µmol/l
Adrenocorticotropin	pg/ml	0.2202	pmol/l
Aldosterone	ng/dl	27.74	pmo/l
Amino acids			
• Alanine	mg/dl	112.2	µmol/l
• α-Aminobutyric acid	mg/dl	96.97	µmol/l
• Arginine	mg/dl	57.40	µmol/l
• Asparagine	mg/dl	75.69	µmol/l
• Aspartic acid	mg/dl	75.13	µmol/l
• Citrulline	mg/dl	57.08	µmol/l
• Cystine	mg/dl	41.61	µmol/l
• Glutamic acid	mg/dl	67.97	µmol/l
• Glutamine	mg/dl	68.42	µmol/l
• Glycine	mg/dl	133.2	µmol/l
• Histidine	mg/dl	64.45	µmol/l
• Hydroxyproline	mg/dl	76.26	µmol/l
• Isoleucine	mg/dl	76.24	µmol/l
• Leucine	mg/dl	76.24	µmol/l
• Lysine	mg/dl	68.40	µmol/l
• Methionine	mg/dl	67.02	µmol/l
• Ornithine	mg/dl	75.67	µmol/l
• Phenylalanine	mg/dl	60.54	µmol/l
• Proline	mg/dl	86.86	µmol/l
• Serine	mg/dl	95.16	µmol/l
• Taurine	mg/dl	79.91	µmol/l
• Threonine	mg/dl	83.95	µmol/l
• Tryptophan	mg/dl	48.97	µmol/l
• Tyrosine	mg/dl	55.19	µmol/l
• Valine	mg/dl	85.36	µmol/l
Amino acid nitrogen	mg/dl	0.7139	mmol/l
Amylase	units/l	1.0	units/l
Androstenedione	µg/l	3.492	nmol/l
Calcitonine	pg/ml	1.0	ng/l
Calcium	mg/dl	0.2495	mmol/l
Calcium ion	meq/l	0.500	mmol/l
Carbone dioxide content	meq/l	1.00	nmol/l
Cholesterol	mg/dl	0.02586	mmol/l
Citrate (as citric acid)	mg/dl	52.05	µmol/l
Cortisol	µg/dl	27.59	nmol/l
C-peptide	ng/ml	0.331	nmol/l
Creatinine	mg/dl	88.40	µmol/l
Creatinine clearance	ml/min	0.01667	ml/s
Cyclic AMP	µg/l	3.038	nmol/l
Cyclic GMP	µg/l	2.897	nmol/l
Dehydroepiandrosterone	µg/l	3.467	nmol/l
Dehydroepiandrosterone sulfate	ng/ml	0.002714	µmol/l
11-Deoxycortisol	µg/dl	28.86	nmol/l
Epinephrine	pg/ml	5.458	pmol/l

	Metric unit	Conversion factor	SI unit
Estradiol	pg/ml	3.671	pmol/l
Estrone	pg/ml	3.699	pmol/l
Fatty acids,nonesterified	mg/dl	0.01	g/l
Follicle-stimulaing hormone	mIU/ml	1.00	IU/l
Fructose	mg/dl	0.05551	mmol/l
Galactose	mg/dl	0.05551	mmol/l
Gases			
• Po_2	mmHg	0.1333	kpa
• Pco_2	mmHg	0.1333	kpa
Gastrin	pg/ml	1.0	ng/l
Gastroinhibitory polypeptide	pg/ml	0.201	pmol/l
Glucagon	pg/ml	1.0	ng/l
Glucose	mg/dl	0.05551	mmol/l
Glycerol,free	mg/dl	0.1086	mmol/l
Growth hormone	ng/ml	1.0	µg/l
β-Hydroxybutyrate (as β-Hydroxybutyric acid)	mg/dl	96.05	µmol/l
17α-Hydroxyprogesterone	µg/l	3.026	nmol/l
Insuline	µU/ml	6.0	pmol/l
Lactate (as lactic acid)	mEq/l	1.0	mmol/l
Lipase	units/l	1.0	units/l
Lipoproteins			
• LDL (as cholesterol)	mg/dl	0.02586	mmol/l
• HDL (as cholesterol)	mg/dl	0.02586	mmol/l
Luteinizing hormone	mIU/ml	1.0	IU/l
Norepinephrine	pg/ml	0.005911	nmol/l
Osmolality	mOsm/kg	1.0	mnol/kg
Pancreatic polypeptide	pg/ml	0.239	pmol/l
Phosphate (as inorganic phosphorus)	mg/dl	0.3229	mmol/l
Phospholipid phosphorus	mg/dl	0.3229	mmol/l
Progesterone	ng/ml	3.180	nmol/l
Prolactine	ng/ml	1.0	µg/l
Protein,total	g/dl	10.0	g/l
Pyruvate(as pyruvic acid)	mg/dl	113.6	µmol/l
Renin	$ng \cdot ml^{-1} \cdot h^{-1}$	0.2778	$ng \cdot l^{-1} \cdot s^{-1}$
Serotonin	µg/dl	0.05675	µmol/l
Somatostatin	pg/ml	0.611	pmol/l
Testosterone	ng/ml	3.467	nmol/l
Thyroid-stimulating hormone	µU/ml	1.0	mU/l
Thyroxine	µg/dl	12.87	nmol/l
Triglycerides	mg/dl	0.0112	mmol/l
Triiodothyronine	ng/dl	0.01536	nmol/l
Urea nitrogen	mg/dl	0.3570	mmol/l
Vasoactive intestinal polypeptide	pg/ml	0.331	pmol/l

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