CONTROVERSIES IN GROWTH HORMONE TREATMENT AND DIAGNOSIS

EDITED BY: Robert Rapaport and Martin Oswald Savage
PUBLISHED IN: Frontiers in Endocrinology and Frontiers in Pediatrics

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-83250-322-5
DOI 10.3389/978-2-83250-322-5

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

CONTROVERSIES IN GROWTH HORMONE TREATMENT AND DIAGNOSIS

Topic Editors:
Robert Rapaport, Icahn School of Medicine at Mount Sinai, United States
Martin Oswald Savage, Queen Mary University of London, United Kingdom

Citation: Rapaport, R., Savage, M. O., eds. (2022). Controversies in Growth Hormone Treatment and Diagnosis. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-83250-322-5

Table of Contents

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 13 September 2022
DOI 10.3389/fendo.2022.1013872

OPEN ACCESS

EDITED AND REVIEWED BY
Sally Radovick,
The State University of New Jersey,
United States

*CORRESPONDENCE
Robert Rapaport
robert.rapaport@mountsinai.org
Martin O. Savage
m.o.savage@qmul.ac.uk

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 07 August 2022
ACCEPTED 09 August 2022
PUBLISHED 13 September 2022

CITATION
Rapaport R and Savage MO (2022) Editorial: controversies in growth hormone treatment and diagnosis. *Front. Endocrinol.* 13:1013872. doi: 10.3389/fendo.2022.1013872

Editorial: Controversies in growth hormone treatment and diagnosis

Robert Rapaport[1*†] and Martin O. Savage[2*†]

[1]Kravis Children's Hospital and Icahn School of Medicine at Mount Sinai, NY, United States, [2]William Harvey Research Institute, Queen Mary, University of London, London, United Kingdom

KEYWORDS

growth, growth hormone, GH deficiency, short stature, GH treatment

Editorial on the Research Topic
Controversies in growth hormone treatment and diagnosis

The diagnosis and treatment of pediatric growth disorders encompasses a wide range of topics including diagnostic criteria, including genetic analysis, indications for therapy with recombinant human growth hormone (rhGH) and efficacy and optimal benefit from this therapy. This Research Topic issue includes 19 articles embracing all of these components and gives a rich account of the current state-of-the-art of growth disorder management. In terms of diagnosis, assessment of GH secretion is a controversial field due to the relative inaccuracy of GH stimulation tests. The value of GH testing is addressed by Yau and Rapaport and by Ibba and Loche who cites evidence of GH deficiency without the need to perform formal stimulation tests. An appraisal of the electronic computer-aided bone age diagnosis system, a key factor in short stature evaluation, is discussed and a high degree of confidence reported in this new technology. The genetic components of growth disorders is widely described by several authors, notably with descriptions of genetic syndromes such as brachydactyly, collagen gene mutations, NPR2 gene variants, GH resistance and ring chromosome 15 syndrome. The response of several of these disorders, including children with idiopathic short stature, to treatment with rhGH is reported. The well known but rarely documented or discussed gender and racial disparities in the evaluation and treatment of short stature and GH Deficiency is addressed in a brief review.

Therapy with rhGH is approved by the FDA and European Medicines Agency (EMA) in GH deficient children and several non-GH deficient disorders such as Turner syndrome and short stature related to birth size small for gestational age. The optimization of this therapy has challenged clinicians since its introduction in 1985. The enhancement of height gain using a combination of rhGH and GnRH analogues to suppress skeletal maturation is elegantly debated by Wit. Two further components of rhGH therapy are safety and adherence to the treatment regimen. Safety is discussed in two articles with reassuring conclusions, one related to all-cause mortality and cancer-risk and the second a broad overview of safety and discussion of the need for long-term clinical surveillance by Cianfarani. The second component of adherence to rhGH therapy

is addressed in a systematic literature review of the data on injectable treatment in a range of chronic conditions and an objective account of patients' perception of the use of the electronic autoinjector Easypod™ which is reported to be associated with high rates of adherence.

The final two articles relate first to the important but rare disorder of severe primary IGF-1 deficiency, or GH resistance, which is approved for treatment with rhIGF-1. The topic discussed is the effect of rhIGF-1 therapy on pubertal timing and growth dynamics with data generated from the European Increlex® Growth Forum Database Registry. Finally, a comprehensive appraisal of current opinions on the effect of long-acting rhGH therapy, which is about to enter clinical paediatric practice, is discussed by Miller.

The Research Topic issue presents balanced, objective and nonpromotional discussions of current controversial topics of clinical relevance. Emphasis is given to developments in genetic diagnosis of rare syndromes, which nevertheless present clinical challenges, and to topical issues such equity in diagnosis and treatment as well as the impact of long-acting rhGH. We are confident that these articles will be of value to clinicians responsible for management of growth disorders and therefore positively impact patient care.

Author contributions

MS drafted initial outline. RR edited and finalized manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers in Endocrinology

MINI REVIEW
published: 10 December 2021
doi: 10.3389/fendo.2021.781044

GH Resistance Is a Component of Idiopathic Short Stature: Implications for rhGH Therapy

Martin O. Savage * *and Helen L. Storr*

Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine & Dentistry, Queen Mary University of London, London, United Kingdom

Idiopathic short stature (ISS) is a term used to describe a selection of short children for whom no precise aetiology has been identified. Molecular investigations have made notable discoveries in children with ISS, thus removing them from this category. However, many, if not the majority of children referred with short stature, are designated ISS. Our interest in defects of GH action, i.e. GH resistance, has led to a study of children with mild GH resistance, who we believe can be mis-categorised as ISS leading to potential inappropriate management. Approval of ISS by the FDA for hGH therapy has resulted in many short children receiving this treatment. The results are extremely variable. It is therefore important to correctly assess and investigate all ISS subjects in order to identify those with mild but unequivocal GH resistance, as in cases of PAPP-A2 deficiency. The correct identification of GH resistance defects will direct therapy towards rhIGF-I rather than rhGH. This example illustrates the importance of recognition of GH resistance among the very large number patients referred with short stature who are labelled as 'ISS'.

Keywords: growth, short stature, growth hormone resistance, genetic defects, idiopathic short stature, growth hormone therapy

OPEN ACCESS

Edited by:
Amit V. Pandey,
University of Bern, Switzerland

Reviewed by:
Alan David Rogol,
University of Virginia, United States
Zvi Laron,
Schneider Children's Medical Center, Israel

***Correspondence:**
Martin O. Savage
m.o.savage@qmul.ac.uk
orcid.org/0000-0001-7902-3376

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *22 September 2021*
Accepted: *09 November 2021*
Published: *10 December 2021*

Citation:
Savage MO and Storr HL (2021) GH Resistance Is a Component of Idiopathic Short Stature: Implications for rhGH Therapy. Front. Endocrinol. 12:781044. doi: 10.3389/fendo.2021.781044

INTRODUCTION

The term idiopathic short stature (ISS) was first applied to short children without a known aetiology over 35 years ago, long before the era of molecular investigation. A current definition of ISS will be discussed below. However, ISS is not a definitive diagnosis. Its original use as a description of children with short stature, who did not have GH deficiency, served a purpose in its day, but now clinicians take the investigation of such children further, with new opportunities presenting a realistic chance of identifying causative pathogeneses (1). As precision medicine attempts to personalise diagnosis and therapy, new genetic discoveries in the GH-IGF-I axis and growth plate chondrogenesis provide opportunities for more precise diagnosis (1).

GH RESISTANCE

GH resistance in children embraces a range of defects in the GH-IGF-I axis characterised by an abnormality in the action of GH (2). In the context of child with short stature, it is the milder forms of GH resistance, which tend to be confused with ISS. The extreme or 'classical' form of GH resistance, so-called Laron syndrome, is relatively easy to diagnose because of its extreme phenotype

and is unlikely to be confused with ISS. However milder or 'non-classical' GH resistance disorders might overlap clinically and thus be mis-categorised as ISS. In 2019, an extensive review of mild or 'non-classical' abnormalities of GH action was published by our group (3). These findings will be summarised below together with a hypothesis that in many cases mild GH resistance disorders may be mis-diagnosed as ISS.

THE ORIGIN OF THE ISS DESIGNATION

The diagnosis of GH deficiency in children entered clinical practice in the late 1960s with the demonstration of GH release following stimulation by insulin-induced hypoglycaemia or acute administration of glucagon and other GH secretagogues (4). GH stimulation tests permitted diagnosis of GH deficiency and thereby separated GH deficient patients from those with similar appearance but normal GH secretion. Towards the end of the era of administration of pituitary-derived hGH, which terminated in 1985 due to the Creutzfeldt-Jakob epidemic, the anticipation of the availability of recombinant hGH (rhGH), first synthesized in 1979 (5), led to short and long-term studies of hGH therapy in subjects with so-called 'normal variant short stature' (6) or labelled as 'short normal' children (7). A conference, convened at the NIH in November 1983 to discuss the future use of rhGH in short children without GH deficiency, concluded that in a society that 'values tallness', controlled research studies of rhGH in such patients were authorized (8). At that meeting, ISS as a diagnostic group acquired scientific respectability.

CURRENT DEFINITION OF ISS AND ITS SUB-CLASSIFICATION

The definition of ISS is clinically important because inclusion of a child with short stature within this designation may, in certain societies where ISS is approved for hGH therapy, provide an indication for this treatment. ISS is currently defined as short stature with height <-2 SDS, normal birth size (birth weight and length >-2 SDS), absence of abnormal physical features and normal general screening investigations, normal body proportions and absence of major dysmorphic features (9). It should be noted that the above definition of ISS is different from the height criterion of <-2.25 SD approved by the FDA for rhGH therapy (10).

The components of ISS were critically appraised in two reviews by Wit et al. in 2008 (10, 11). A Consensus Statement on ISS management was also published in 2008 (12). ISS was subdivided into familial short stature (FSS) with normal or delayed bone age and non-familial short stature (NFSS) with normal or delayed bone age (9–11). The definitions proposed for FSS and NFSS are based on the calculation of 'conditional' target height (cTH), which is adjusted for the correlation between maternal and paternal heights, so-called assortative mating, and for the correlation between children's height SDS and mid-parental height SDS (13). The definition of FSS is Height SDS = cTH SDS ± 1.6 and of NFSS, Height SDS < cTH SDS -1.6 based on the fact that 95% of healthy children have Height SDS = cTH SDS ± 1.6 (the TH range). It should be noted that FSS may co-exist with constitutional delay of growth and puberty (CDGP) in the same patient, who might present earlier with short stature. Most children with CDGP seen in a growth disorders clinic have at least one parent who is short.

It is likely that in most subjects with FSS, the short stature is related to the inheritance of polygenic variants from both parents with multiple small negative effects on height. However, a copy number variant (CNV) or monogenic defect is also possible, particularly if there is a pattern of dominant inheritance, notably from one parent. The inheritance of multiple variants in the same or different growth-related pathways may occur (14). In children with NFSS and a slow tempo of growth constitutional delay of growth and puberty is statistically the most likely diagnosis, particularly if bone age is delayed and the family history is positive for delayed puberty. However, also a recessive or *de novo* pathogenic gene variant or CNV should be considered. It is in the NFSS group that defects associated with adult height below parental target height are most likely to occur.

At the time when the GH-IGF-I axis was considered to be the major influence for growth regulation, 'ISS' was used to describe children who fell between GH deficiency and GH insensitivity in the so-called GH-IGF-I axis continuum model (**Figure 1**) (15). According to this model, ISS subjects should have a normal physiological equilibrium between GH sensitivity and GH deficiency, which is the case for those with FSS, where no endocrine defect in the child or parents has been identified. However, since the discovery that most genes associated with normal linear growth have no direct relationship with the GH-IGF-I axis (16), it appears more useful to think in terms of another conceptual framework for understanding short and tall stature that is centred not on the GH-IGF-I axis, but rather on the growth plate (17). In the 21st Century, 35 years after its inception, ISS therefore describes a highly heterogeneous group of short patients and should no longer be used as a single definitive diagnostic category (1).

ASSESSMENT OF PATIENTS WITH SHORT STATURE AND INVESTIGATION OF ISS AND GH RESISTANCE

A diagnostic algorithm for investigation of short stature is shown in **Figure 2**. The three key approaches of clinical assessment, endocrine evaluation and genetic analysis should have equal status in the hierarchy of assessment variables. We resist the suggestion to give genetic analysis more prominence (14) at the expense of clinical assessment, because clinical skills are crucial in terms of identifying a phenotype and taking a valuable history (1). The classification of growth disorders into primary growth

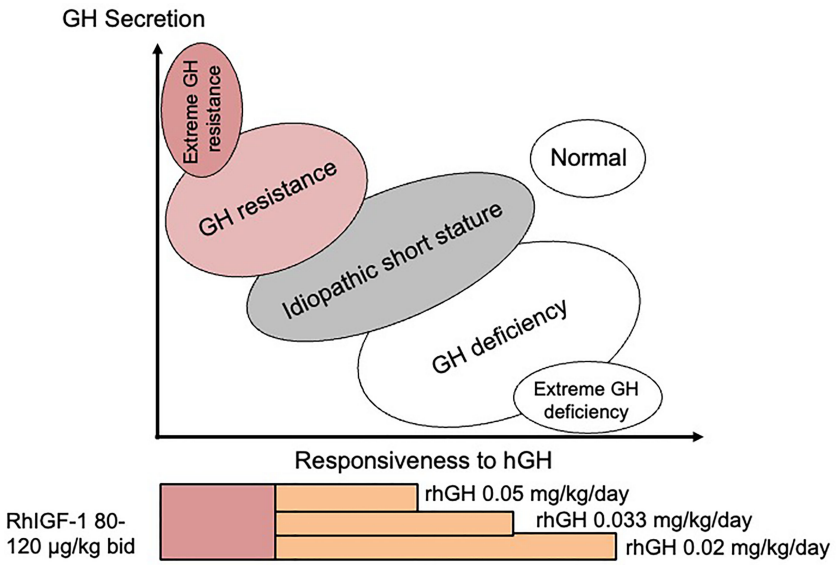


FIGURE 1 | The continuum model showing the relationship between disorders of the GH-IGF-I axis and responsiveness to hGH therapy. Suggested therapy with doses of rhGH and rhIGF-I relating to the different disorders is also shown.

plate defects and secondary abnormalities affecting growth plate function has redressed the balance of probability of correct pathogenesis away from the GH-IGF-I axis towards defects of chondrogenesis (1, 17). A diagnosis of GH resistance can be made following evaluation of GH secretion and the IGF system (**Figure 2**), however the precise molecular pathogenesis will require next generation sequencing using either candidate gene or whole exome sequencing techniques (1, 2, 14).

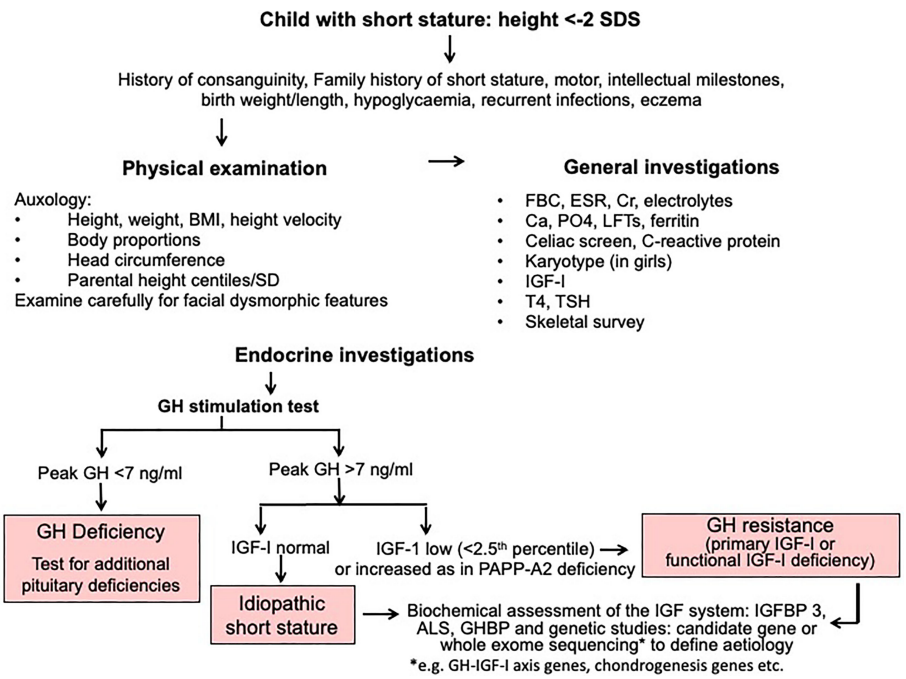


FIGURE 2 | Algorithm for investigation of short stature, idiopathic short stature and GH resistance.

ENDOCRINE ABNORMALITIES IN PATIENTS INITIALLY CONSIDERED TO HAVE ISS

In the 1980s and 1990s the study of childhood linear growth focused on the function of different components of the GH-IGF-I axis and enormous progress in the understanding of this axis was made (18). The original somatomedin hypothesis, published in 1957 (19), was up-dated 50 years later (20) showing that the IGF system played a key role in growth regulation with both circulating and peripherally produced IGF-I having individual roles (21). IGF-I deficiency was reported to occur in a proportion of short patients with normal GH secretion (22), which placed some ISS patients in an intermediate position between GH deficiency and GH resistance, although some overlap existed.

GH RESISTANCE AS A COMPONENT OF ISS

Evidence has accumulated that some ISS patients have a degree of functional GH resistance (22) with a broad range of generation of IGF-I in response to GH. The important study by Cohen et al. in 2007 reported that in some ISS patients high doses of rhGH were needed to reach a serum IGF-I concentration of +2 SD (23). Evidence of subnormal generation of IGF-I was also demonstrated in the elegant studies by Buckway and Selva of responses in the IGF-I generation test (IGFGT). Compared to normal control subjects, ISS patients had basal IGF-I levels in the lower half of the normal range and after GH stimulation on days 5 and 8 of the IGFGT, IGF-I levels were significantly lower than normal, regardless of GH dose (24, 25). It should be noted that the IGFGT has suffered from a lack of standardization, both of dose of rhGH used and duration of stimulation (10). Similarly, normative data have not been established and the routine use of the IGFGT is not recommended in the investigation of ISS (12). The hormonal findings of possible IGF-I deficiency in ISS challenge the definition that states that ISS is associated with no endocrine abnormality. The molecular basis of these findings was not apparent at that time.

VARIANTS IN GENES REGULATING GH ACTION WITH PHENOTYPES CONSISTENT WITH ISS

ISS patients may have variable GH resistance and IGF-I concentrations (10) and consistent with this, a proportion have a diminished response to rhGH therapy (11, 15). Therefore, it has been suggested that less deleterious *GHR* gene defects may cause ISS associated with features of GH resistance (26). Numerous studies of ISS cohorts have reported heterozygous *GHR* variants occurring with a frequency ranging from 5 to 15.5% (27). It has also been noted that *GHR* sequence changes are common in children with ISS with many also identified in control subjects and normal stature family members (3).

Since the inception of genetic investigations of short stature phenotypes in the late 1980s, a number of pathogenic variants have been discovered in children labelled as having ISS. Mild forms of GH resistance can be broadly divided into three categories; 1) aberrations of GH signalling caused by homozygous or heterozygous variants of genes encoding the GH receptor (*GHR*) or Signal Transducer And Activator of Transcription 5B (*STAT5B*) (3, 28); 2) defects of IGF-I secretion (*IGFI*), transport (*IGFALS*) and bioavailability (*PAPPA2*) (3, 29, 30) and; 3) IGF-I insensitivity (*IGFIR*) (31). A summary of phenotypic and endocrine features of genetic defects consistent with ISS is shown in **Table 1**.

GHR MUTATIONS ASSOCIATED WITH MILD PHENOTYPES

In the endocrine scientific community, there was initially some resistance to the concept of *GHR* mutations being associated with a milder phenotype than is seen in classical Laron syndrome. However, there is clear evidence that GH insensitivity of primary genetic origin is not always associated with extreme short stature (3). Evidence of a phenotypic and biochemical continuum in patients characterised as having GH insensitivity first arose from a European cohort of 82 subjects identified for replacement therapy with rhIGF-I (2). Phenotypic characteristics were strikingly broad with heights ranging from -2.2 to -10.4 SDS. An analysis of 70 subjects with features of GH insensitivity and *GHR* mutations in our laboratory showed that height SDS was significantly related to the type of *GHR* defect, with subjects having dominant negative or homozygous 6ψ pseudoexon mutations (see below) being more mildly affected than those with nonsense, missense or splice mutations (2).

Dominant Negative GHR Mutations

Heterozygous dominant-negative *GHR* mutations were reported in seven children with growth failure and heights ranging from -2.0 to -4.2 SDS (3, 32). In addition to the short stature being milder than in Laron syndrome, none of these patients had the dysmorphic cranio-facial features of classical GH insensitivity. Facial appearances were normal or only minimally dysmorphic. All subjects had IGF-I deficiency with normal GH secretion as evidence of their GH resistance. It is understandable that such patients, if not investigated in detail, could be incorrectly categorised as having ISS and therefore potentially treated with rhGH, to which they would not be responsive (11).

The Intronic GHR Pseudoexon Mutation

The intronic *GHR* pseudoexon mutation (6ψ) was first described in 2001 in four siblings with mild GH insensitivity from a consanguineous Pakistani family (33). The inclusion of the abnormal pseudoexon sequence in the *GHR* transcript translates to the insertion of 36 new amino acids within the extracellular domain and induces abnormal function of the

TABLE 1 | Summary of phenotypic and biochemical features of defects causing GH resistance originally labelled as ISS.

Phenotype	Gene defect						
	GHR heterozygous dominant negative	*GHR pseudo-exon*	*STAT5B heterozygous dominant negative*	*IGFI*	*IGF2 (heterozygous variants)*	*IGFALS*	*PAPPA2*
Short stature	+	+	+	+	+	+	+
Mid-face hypoplasia	–	*	+/–	–	–	–	–
Other facial dysmorphism	–	–	–	+ Micrognathia	+	–	+ Long thin nose Small chin
Deafness	–	–	–	+/–	–	–	–
Microcephaly	–	–	–	+	–	–	+/–
Intellectual deficits	–	–	–	+	–	–	–
Pubertal delay	–	–	+/–	–	–	+	–
Immune deficiency	–	–	+	–	–	–	–
Hypoglycemia	–	+	–/+	–	n/r	–	–
Hyper-insulinemia	–	–	–	+/–	n/r	+	+
IGF-I	↓	n/↓	↓	n/↓	n/↑	↓	↑
IGFBP-3	↓	n/↓	↓	n	n/↑	↓	↑
ALS	n/↓	n/↓	+/–	n	n/r	↓	↑
GH	↑	n/↑	↑	n/↑	n/↑	↑	↑
GHBP deficiency	+/–	–	–	–	–	–	–

*+, Positive; –, Negative; +/–, Predominantly positive; –/+, Predominantly negative; *, approximately 50%; n/a, not applicable; n/r, not reported; ↑, increased; ↓, decreased; n, normal; ALS, acid labile subunit; IGFBP-3, IGF binding protein-3; GHBP, growth hormone binding protein.*

mutant GHR protein. In 2018, the group from the William Harvey Research Institute in London reported 20 cases, the clear majority being from consanguineous Pakistani families (34). The mean height SDS was -4.1 ± 0.95, mean IGF-I SDS was -2.8 ± 1.4 SDS and mean IGFBP-3 SDS was -3.0 ± 2.1. Ten out of the 20 subjects (50%) had facial features consistent with classical GH insensitivity and 10 had normal facial appearance. The phenotype of 6ψ subjects is therefore variable, more so than in other extracellular *GHR* mutations and it has recently been reported that variable amounts of 6ψ- and wild type-*GHR* transcripts were identified in 6ψ patients. Higher 6ψ:wild-type *GHR* transcript ratio correlated with the severity of the short stature phenotype (35).

IGFALS and PAPP-A2 Mutations

Additional homozygous mutations associated with relatively mild short stature, hence predisposing to incorrect categorisation as ISS, are defects of the acid-labile subunit (*IGFALS)* and pregnancy-associated plasma protein A2 (*PAPP-A2)* genes. Both these proteins have key functional roles in the transport of IGF-I and IGFBP-3 in the circulation and the regulation of IGF-I bioavailability to peripheral tissues. *IGFALS* mutations cause severe deficiency of circulating ALS, with the inability to form the ternary complex resulting in marked reduction of serum IGF-I and IGFBP-3 concentrations (2, 3). However, paradoxically, the degree of growth disturbance is mild, presumably related to generation of free IGF-I in peripheral tissues, hence preserving autocrine/paracrine IGF-I action.

In 2016, the first human cases of PAPP-A2 deficiency were described that led to increased circulating IGF-I and IGFBP-3 concentrations due to an inability of the PAPP-A2 protein to cleave the ternary complex and release free bioavailable IGF-I (29). As in ALS deficiency, the phenotype is subtle, being characterised by mild short stature (height ranging from -1.0 to -3.8 SDS), mild microcephaly, long thin nose and small chin with long fingers and toes. However, the serum concentrations of (total) IGF-I, IGFBP-3 and ALS are diagnostically elevated (3).

Heterozygous Mutations Causing Short Stature

Heterozygous mutations of key genes regulating GH action may be associated with short stature phenotypes, but these are less severe than those caused by their homozygous counterparts. Examples are mutations of the following genes; *GHR*, *STAT5B*, *IGF-I* and *IGFALS* (1, 3).

ISS AS AN INDICATION FOR RHGH THERAPY

When hGH was approved by the FDA in October 1985 for therapy in children with 'inadequate GH', this was contingent on the establishment of post-marketing surveillance. Genentech, the makers of the approved rhGH, Protropin, set up the National Cooperative Growth Study (NCGS), within which ISS was a designated diagnostic group (36). 'Idiopathic short stature', thus became an established label for short children with normal GH secretion, normal birth weight and absence of chromosomal defects or chronic illness and was soon adopted throughout the paediatric endocrinology community, particularly in the USA. Alternative terms were proposed such as idiopathic growth failure (IGF) or growth failure of unknown aetiology (GFUE) to change the emphasis from "stature" to "growth" or growth rate (1). However, the term ISS prevailed and thirty five years

later, remains a popular designation for short children with no defined aetiology. Importantly, patients with "normal variant short stature" specifically those with familial short stature and constitutional growth delay were not excluded from the "ISS" designation.

Randomized clinical trials with rhGH were led by the Pharma Industry and produced positive growth-promoting results (37, 38) and ISS was soon referred to as '*a condition termed idiopathic short stature*' or a '*diagnostic category*' (39). Positive data confirmed the effects of rhGH therapy, notably compared with placebo-treated controls (38). Predictably, these results lead to approval of ISS by the FDA in 2003 as an indication for rhGH therapy, under the criteria of height <-2.25 SD without evidence of underlying disease or GH deficiency and short expected adult height. This decision had major implications on clinical practice as suddenly 400,000 children in the USA were eligible for rhGH therapy (40). Similar applications to the European Medicines Agency were unsuccessful, due largely to the absence of data showing a positive rhGH-effect on quality of life (41).

The FDA approval for hGH therapy of ISS consolidated this category of patients in the minds of paediatricians with data on efficacy and safety accumulating in international databases such as the Kabi international growth study (KIGS) and NCGS (42). Results of cohorts of ISS subjects were, and still are, being regularly analysed and published (43, 44) and are used as the basis for management guidelines (45). ISS is also used as a diagnostic category in the ESPE and International Classifications of Pediatric Endocrine Disorders (www.icped.org) (46). Notably, the ISS patients treated with rhGH responded inconsistently and in particular, growth during year 1 of therapy did not predict the response in year 2, which emphasised the marked heterogeneity of patients carrying the ISS label (47).

INFLUENCE OF GH RESISTANCE ON GROWTH RESPONSE TO RHGH

In clinical medicine, 'diagnosis' generally means that the aetiology of a condition has been identified. As stated above, ISS is not a final diagnosis and the designation 'idiopathic' means that no aetiology has been identified. We accept that the term ISS will continue to be used. The FDA licence for rhGH therapy in ISS increases the temptation for clinicians to use this label in order to prescribe rhGH either as short-term or long-term therapy. Doses of rhGH higher than those recommended by the FDA and EMA must not be used as this would increase the risk of adverse effects of supranormal IGF-I levels and possible non-growth related effects such as acromegaloid features (11).

However, this approach can be both psychologically damaging when an over-optimistic height prognosis is predicted to rhGH therapy and counter-productive when a pathogenesis supporting alternative therapy such as rhIGF-I would be more effective. The challenge for a clinician who is presented with a patient with short stature, who has normal GH secretion, but deserves rhGH therapy because of significant short stature, revolves around decisions of whether to treat with rhGH doses appropriate for GH deficiency or to use higher doses advised for ISS. Alternatively, should rhIGF-I be prescribed based on evidence of GH resistance?

Treatment with rhGH is usually taken as the 'safest choice', but may not be the best choice if the diagnosed defect is situated in a position on the continuum model GH responsiveness scale which suggests that rhGH therapy is unlikely to be beneficial (**Figure 1**). Clinicians have been hesitant to prescribe rhIGF-I as first choice in this situation. Published data on the effect of rhIGF-I in ISS subjects are extremely rare. WE would recommend that rhIGF-I is given for specific disorders where the origin and nature of the Gh resistance has been clearly demonstrated. However, if serum IGF-I is consistently low and GH secretion is normal, a diagnosis of GH resistance can be made and first-line therapy with rhIGF-I is indicated, which can be beneficial in the long-term (45).

A case in point is the molecular disorder of *PAPP-A2* mutations. Affected children have mild short stature with subtle dysmorphic features. If labelled as ISS, rhGH therapy might well be prescribed. In fact, identifying and understanding the correct pathogenesis will lead to therapy with rhIGF-I, as the mutation results in deficiency of free IGF-I, and positive responses to rhIGF-I therapy have now been reported (48, 49). The same argument applies to mild *GHR* mutations, where responses to rhIGF-I have also been documented (3, 34), in contrast to lack of evidence of responses to rhGH. Genetic identification of *IGFIR* defects can be compared to published experience of rhGH therapy in such patients (31) rather than to non-specific responses to rhGH in idiopathic SGA subjects.

CONCLUSIONS

We have described how the term 'idiopathic short stature' was created, then adopted and is likely to continue to be used. Investigation of children with short stature should in our opinion follow three simultaneous lines of approach, clinical assessment, endocrine evaluation and genetic analysis. These three components should have equal status in the hierarchy of the investigational tree. This comprehensive diagnostic approach in children with short stature and normal GH secretion can give a relatively high positive diagnostic yield (50). The identification of GH resistance in a child who would otherwise be labelled as having ISS, immediately removes the patient from the ISS category and treatment can be approached on an individual basis. If rhGH is to be used for the treatment of ISS, a high dose of rhGH ~50 μg/kg/day should be used and if no growth acceleration is seen after one year, the treatment should be discontinued and alternative therapy considered (12).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

1. Rapaport R, Wit JM, Savage MO. Growth Failure: 'Idiopathic' Only After a Detailed Diagnostic Evaluation. *End Conn* (2021) 10:R1–14. doi: 10.1530/EC-20-0585
2. David A, Hwa V, Metherell LA, Netchine I, Camacho-Hübner C, Clark AJL, et al. Evidence for a Continuum of Genetic, Phenotypic and Biochemical Abnormalities in Children With Growth Hormone Insensitivity. *Endocr Rev* (2011) 32:472–97. doi: 10.1210/er.2010-0023
3. Storr HL, Chatterjee S, Metherell LA, Foley C, Rosenfeld RG, Backeljauw PF, et al. Nonclassical GH Insensitivity: Characterization of Mild Abnormalities of GH Action. *EndocriRev* (2019) 40:476–505. doi: 10.1210/er.2018-00146
4. Frasier SD. The Serum Growth-Hormone Response to Hypoglycemia in Dwarfism. *J Pediatr* (1967) 71:625–38. doi: 10.1016/s0022-3476(67)80197-5
5. Goeddel DV, Heyneker HL, Hozumi T, Arentzen R, Itakura K, Yansura DG, et al. Direct Expression in Escherichia Coli of a DNA Sequence Coding for Human Growth Hormone. *Nature* (1979) 281:544–8. doi: 10.1038/281544a0
6. Rudman D, Kutner MH, Blackston RD, Cushman RA, Bain RP, Patterson JH. Children With Normal-Variant Short Stature: Treatment With Human Growth Hormone for Six Months. *N Eng J Med* (1981) 305:123–31. doi: 10.1056/NEJM198107163050302
7. Van Vliet G, Styne DM, Kaplan SL, Grumbach MM. Growth Hormone Treatment for Short Stature. *N Eng J Med* (1983) 309:1016–22. doi: 10.1056/NEJM198310273091703
8. Underwood LE. Report of the Conference on Uses and Possible Abuses of Biosynthetic Human Growth Hormone. *N Eng J Med* (1984) 311:606–8. doi: 10.1056/NEJM198408303110925
9. Ranke MB. Towards a Consensus on the Definition of Idiopathic Short Stature. *Horm Res* (1996) 45(Suppl 2):64–6. doi: 10.1159/000184851
10. Wit JM, Clayton PE, Rogol AD, Savage MO, Saenger PH, Cohen P. Idiopathic Short Stature: Definition, Epidemiology, and Diagnostic Evaluation. *GH IGF Res* (2008) 18:89–110. doi: 10.1016/j.ghir.2007.11.004
11. Wit JM, Reiter EO, Ross JL, Saenger PH, Savage MO, Rogol AD, et al. Idiopathic Short Stature: Management and Growth Hormone Treatment. *GH IGF Res* (2008) 18:111–35. doi: 10.1016/j.ghir.2007.11.003
12. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, et al. Consensus Statement on the Diagnosis and Treatment of Children With Idiopathic Short Stature: A Summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab* (2008) 93:4210–7. doi: 10.1210/jc.2008-0509
13. Hermanussen M, Cole J. The Calculation of Target Height Reconsidered. *Horm Res* (2003) 59:180–3. doi: 10.1159/000069321
14. Dauber A. Genetic Testing for the Child With Short Stature-Has the Time Come To Change Our Diagnostic Paradigm? *J Clin Endocrinol Metab* (2019) 104:2766–9. doi: 10.1210/jc.2019-00019
15. Savage MO, Burren CP, Rosenfeld RG. The Continuum of Growth Hormone-IGF-I Axis Defects Causing Short Stature: Diagnostic and Therapeutic Challenges. *Clin Endocrinol (Oxf)* (2010) 72:721–8. doi: 10.1111/j.1365-2265.2009.03775
16. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, et al. GIANT Consortium Meta-Analysis of Genome-Wide Association Studies for Height and Body Mass Index in ∼700000 Individuals of European Ancestry. *Hum Mol Genet* (2018) 27:3641–9. doi: 10.1093/hmg/ddy271
17. Baron J, Sävendahl L, De Luca F, Dauber A, Phillip M, Wit JM, et al. Short and Tall Stature: A New Paradigm Emerges. *Nat Rev Endocrinol* (2015) 11:735–46. doi: 10.1038/nrendo.2015.165
18. Blum WF, Alherbish A, Alsagheir A, El Awwa A, Kaplan W, Koledova E, et al. The Growth Hormone-Insulin-Like Growth Factor-I Axis in the Diagnosis and Treatment of Growth Disorders. *Endocr Conn* (2018) 7:R212–22. doi: 10.1530/EC-18-0099
19. Salmon WD, Daughaday WH. A Hormonally Controlled Serum Factor Which Stimulates Sulfate Incorporation by Cartilage *In Vitro*. *J Lab Clin Med* (1957) 49:825–36.
20. Kaplan SA, Cohen P. The Somatomedin Hypothesis 2007: 50 Years Later. *J Clin Endocrinol Metab* (20072007) 92:4529–35. doi: 10.1210/jc.2007-0526
21. LeRoith D. Clinical Relevance of Systemic and Local IGF-I: Lessons From Animal Models. *Pediatr Endocrinol Rev* (2008) 5(Suppl 2):739–43.
22. Park P, Cohen P. Insulin-Like Growth Factor (IGF-I) Measurements in Growth Hormone (GH) Therapy of Idiopathic Short Stature (ISS). *GH IGF Res* (2005) 15:S13–20. doi: 10.1016/j.ghir.2005,06.011
23. Cohen P, Rogol AD, Howard CP, Bright GM, Kappelgaard AM, Rosenfeld RG. American Norditropin Study Group. Insulin Growth Factor-Based Dosing of Growth Hormone Therapy in Children: A Randomized, Controlled Study. *J Clin Endocrinol Metab* (2007) 92:2480–6. doi: 10.1210/jc.2007-0204
24. Buckway CK, Guevara-Aguirre J, Pratt KL, Burren CP, Rosenfeld RG. The IGF-I Generation Test Revisited: A Marker of GH Sensitivity. *J Clin Endocrinol Metab* (2001) 86:5176–83. doi: 10.1210/jcem.86.11.8019
25. Selva KA, Buckway CK, Sexton G, Pratt KL, Tjoeng E, Guevara-Aguirre J, et al. Reproducibility in Patterns of IGF Generation With Special Reference to Idiopathic Short Stature. *Horm Res* (2003) 60:237–46. doi: 10.1159/000074038
26. Pedicelli S, Peschiaroli E, Violi E, Cianfarani S. Controversies in the Definition and Treatment of Idiopathic Short Stature (ISS). *J Clin Res Pediatr Endocrinol* (2009) 1:105–15. doi: 10.4008/jcrpe.v1i3.53
27. Sjoberg M, Salazar T, Espinosa C, Dagnino A, Avila A, Eggers M, et al. Study of GH Sensitivity in Chilean Patients With Idiopathic Short Stature. *J Clin Endocrinol Metab* (2001) 86:4375–81. doi: 10.1210/jcem.86.9.7850
28. Klammt J, Neumann D, Gevers EF, Andrew SF, Schwartz ID, Rockstroh D, et al. Dominant-Negative STAT5B Mutations Cause Growth Hormone Insensitivity With Short Stature and Mild Immune Dysregulation. *Nat Comm* (2018) 9:2105. doi: 10.1038/s41467-018-04521-0
29. Dauber A, Muñoz-Calvo MT, Barrios V, Domené HM, Kloverpris S, Serra-Juhé C, et al. Mutations in Pregnancy-Associated Plasma Protein A2 Cause Short Stature Due to Low IGF-I Availability. *EMBO Mol Med* (2016) 8:363–74. doi: 10.15252/emmm.201506106
30. Işık E, Haliloglu B, van Doorn J, Demirbilek H, Scheltinga SA, Losekoot M, et al. Clinical and Biochemical Characteristics and Bone Mineral Density of Homozygous, Compound Heterozygous and Heterozygous Carriers of Three NovelMutations. *Eur J Endocrinol* (2017) 176:657–67. doi: 10.1530/EJE-16-0999
31. Walenkamp MJE, Robers JML, Wit JM, Zandwijken GRJ, van Duyvenvoorde HA, Oostdijk W, et al. Phenotypic Features and Response to GH Treatment of Patients With a Molecular Defect of the IGF-I Receptor. *J Clin Endocrinol Metab* (2019) 104:3157–71. doi: 10.1210/jc.2018-02065
32. Vairamani K, Merjaneh L, Casano-Sancho P, Sanli ME, David A, Metherell LA, et al. Novel Dominant-Negative GH Receptor Mutations Expands the Spectrum of GHI and IGF-I Deficiency. *J Endocr Soc* (2017) 1:345–58. doi: 10.1210/js.2016-1119
33. Metherell LA, Akker SA, Munroe PB, Rose SJ, Caulfield M, Savage MO, et al. Pseudoexon Activation as a Novel Mechanism for Disease Resulting in Atypical Growth-Hormone Insensitivity. *Am J Hum Genet* (2001) 69:641–6. doi: 10.1086/323266
34. Chatterjee S, Shapiro L, Rose SJ, Mushtaq T, Clayton PE, Ten SB, et al. Phenotypic Spectrum and Responses to Recombinant Human IGF-I (rhIGF-I) Therapy in Patients With Homozygous Intronic Pseudoexon Growth Hormone Receptor Mutations. *Eur J Endocrinol* (2018) 178:481–9. doi: 10.1530/EJE-18-0042
35. Chatterjee S, Cottrell E, Rose SJ, Mushtaq T, Maharaj AV, Williams J, et al. Growth Hormone Receptor (GHR) Gene Transcript Heterogeneity may Explain Phenotypic Variability in Patients With Homozygous GHR Pseudoexon (6Ψ) Mutation. *Endocr Conn* (2020) 9:211–22. doi: 10.1530/EC-20-0026
36. Hintz RL. The Importance of the National Cooperative Growth Study (NCGS). In: Carel J-C, editor. *Deciphering Growth*. Berlin Heidelberg: Springer-Verlag (2005). p. 131–41.
37. Hintz RL, Attie KM, Baptista J, Roche A. Effect of Growth Hormone Treatment on Adult Height of Children With Idiopathic Short Stature. *N Eng J Med* (1999) 340:502–7. doi: 10.1056/NEJM199902183400702
38. Leschek EW, Rose SR, Yanovski JA, Troendle JF, Quigley CA, Chipman JJ, et al. National Institute of Child Health and Human Development-Eli Lilly & Co. Growth Hormone Collaborative Group. *J Clin Endocrinol Metab* (2004) 89:3140–8. doi: 10.1210/jc.2003-031457
39. Cohen P. Controversy in Clinical Endocrinology: Problems With Reclassification of Insulin-Like Growth Factor I Production and Action

Disorders. *J Clin Endocrinol Metab* (2006) 91:4235–6. doi: 10.1210/jc.2006-1641
40. Swatz Topor L, Feldman HA, Bauchner H, Cohen L. Variation in Methods of Predicting Adult Height for Children With Idiopathic Short Stature. *Pediatrics* (2010) 126:937–44. doi: 10.1542/peds.2009-3649
41. Ranke MB, Wit JM. Growth Hormone - Past, Present and Future. *Nat Rev Endocrinol* (2018) 14:285–300. doi: 10.1038/nrendo.2018.22
42. Ranke MB, Lindberg A, Price DA, Darendeliler F, Albertsson-Wikland K, Wilton P, et al. KIGS International Board. Age at Growth Hormone Therapy Start and First-Year Responsiveness to Growth Hormone Are Major Determinants of Height Outcome in Idiopathic Short Stature. *Horm Res* (2007) 68:53–62. doi: 10.1159/000098707
43. Kaplowitz PB, Shulman DI, Frane JW, Jacobs J, Lippe B. Characteristics of Children With Best and Poorest First- and Second-Year Growth During rhGH Therapy: Data From 25years of the Genentech National Cooperative Growth Study (NCGS). *Int J Pediatr Endocrinol* (2013) 9:2013–9. doi: 10.1186/1687-9856-2013-9
44. Sävendahl L, Polak M, Backeljauw P, Blair J, Miller BS, Rohrer TR, et al. Treatment of Children With GH in the United States and Europe: Long-Term Follow-Up From NordiNet® IOS and ANSWER Program. *J Clin Endocrinol Metab* (2019) 104:4730–42. doi: 10.1210/jc.2019-00775
45. Grimberg A, Allen DB. Growth Hormone Treatment for Growth Hormone Deficiency and Idiopathic Short Stature: New Guidelines Shaped by the Presence and Absence of Evidence. *Curr Opin Pediatr* (2017) 29:466–71. doi: 10.1097/MOP.0000000000000505
46. *International Classification of Pediatric Endocrine Diagnoses* (2016). Available at: www.icped.org.
47. Deodati A, Cianfarani S. Impact of Growth Hormone Therapy on Adult Height of Children With Idiopathic Short Stature: Systematic Review. *Br Med J* (2011) 342:c7157. doi: 10.1136/bmj.c7157
48. Cabrera-Salcedo C, Mizuno T, Tyzinski L, Andrew M, Vinks AA, Frystyk J, et al. Pharmacokinetics of IGF-I in PAPP-A2-Deficient Patients, Growth Response, and Effects on Glucose and Bone Density. *J Clin Endocrinol Metab* (2017) 102:4568–77. doi: 10.1210/jc.2017-01411
49. Muñoz-Calvo MT, Barrios V, Pozo J, Chowen JA, Martos-Moreno GÁ, Hawkins F, et al. Treatment With Recombinant Human Insulin-Like Growth Factor-1 Improves Growth in Patients With PAPP-A2 Deficiency. *J Clin Endocrinol Metab* (2016) 101:3879–83. doi: 10.1210/jc.2016-2751
50. Andrews A, Maharaj A, Cottrell E, Chatterjee S, Shah P, Denvir L, et al. Genetic Characterization of Short Stature Patients Presenting With Phenotypic and Endocrine Overlap of Known Growth Hormone Insensitivity Syndromes. *J Clin Endocrinol Metab* (2021) 106:e4716–33. doi: 10.1210/clinem/dgab437

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Endocrinology

REVIEW
published: 16 December 2021
doi: 10.3389/fendo.2021.812196

Should Skeletal Maturation Be Manipulated for Extra Height Gain?

*Jan M. Wit**

Division of Pediatric Endocrinology, Department of Pediatrics, Willem-Alexander Children's Hospital, Leiden University Medical Center, Leiden, Netherlands

Skeletal maturation can be delayed by reducing the exposure to estrogens, either by halting pubertal development through administering a GnRH analogue (GnRHa), or by blocking the conversion of androgens to estrogens through an aromatase inhibitor (AI). These agents have been investigated in children with growth disorders (off-label), either alone or in combination with recombinant human growth hormone (rhGH). GnRHa is effective in attaining a normal adult height (AH) in the treatment of children with central precocious puberty, but its effect in short children with normal timing of puberty is equivocal. If rhGH-treated children with growth hormone deficiency or those who were born small-for-gestational age are still short at pubertal onset, co-treatment with a GnRHa for 2-3 years increases AH. A similar effect was seen by adding rhGH to GnRHa treatment of children with central precocious puberty with a poor AH prediction and by adding rhGH plus GnRHa to children with congenital adrenal hyperplasia with a poor predicted adult height on conventional treatment with gluco- and mineralocorticoids. In girls with idiopathic short stature and relatively early puberty, rhGH plus GnRHa increases AH. Administration of letrozole to boys with constitutional delay of growth puberty may increase AH, and rhGH plus anastrozole may increase AH in boys with growth hormone deficiency or idiopathic short stature, but the lack of data on attained AH and potential selective loss-of-follow-up in several studies precludes firm conclusions. GnRHas appear to have a good overall safety profile, while for aromatase inhibitors conflicting data have been reported.

Keywords: growth, skeletal maturation, bone age, adult height, aromatase inhibitors, GnRH analogue, growth hormone, predicted adult height

OPEN ACCESS

Edited by:
M. Savage,
Queen Mary University of London,
United Kingdom

Reviewed by:
Stefano Cianfarani,
University of Rome Tor Vergata, Italy
Jin Soon Hwang,
Ajou University, South Korea

***Correspondence:**
Jan M. Wit
j.m.wit@lumc.nl

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *09 November 2021*
Accepted: *23 November 2021*
Published: *16 December 2021*

Citation:
Wit JM (2021) Should Skeletal Maturation Be Manipulated for Extra Height Gain? Front. Endocrinol. 12:812196. doi: 10.3389/fendo.2021.812196

1 INTRODUCTION

There are three therapeutic approaches to increase adult height (AH) for a child with a growth disorder. First, recombinant human growth hormone (rhGH) has been approved for several causes of short stature in children, and in most conditions this treatment (if initiated at a young age) results in an AH within the genetic target height range of the patient. Second, experiments of nature have suggested that keeping the estrogen exposure low in adolescence might delay skeletal maturation and increase AH, which has led to clinical studies on the efficacy and safety of two forms of medication aimed at reducing estrogen exposure, i.e. gonadotropin-releasing hormone (GnRH) analogues (GnRHas) and aromatase inhibitors (AIs). Third, also the combination of rhGH and a GnRHa or AI has been investigated,

particularly if the child's height is still low at onset of puberty. This approach is based on the hypothesis that decreasing estrogen exposure slows skeletal maturation and thereby prolongs the time during which rhGH can stimulate linear growth, and thus results in increased AH. In the present paper I review the available clinical data of the second and third approaches.

Multiple "expert opinions" have been published on this topic [e.g. (1–4) in the last 5 years], including two from our own group (5, 6). The appearance of many reports in the literature describing the effect of GnRHas or AIs in patients with various forms of growth failure, suggests that these compounds are widely used, despite their uncertain efficacy and safety profile, as well as their off-label status for these indications.

One could wonder why one would spend time to produce yet one more "expert opinion" on this topic, while in virtually all classifications of the levels of evidence (7) the expert opinion is considered to be in the lowest grade of scientific value. The answer is threefold. First, I believe that the cumulative observations of published reports (mainly offering a low grade of evidence when assessed separately) on the use of GnRHas for different specific conditions may still lead to general conclusions on their efficacy in growth disorders. This implies that a detailed analysis of strengths and weaknesses of all published data is warranted, followed by a general assessment. Second, it is unlikely that large randomized controlled trials (RCTs) continuing until AH will ever be carried out on these interventions in the various growth conditions, so that I fear that for the next decade the level of evidence will remain as low as it currently is. This implies that it is worthwhile to make an effort to extract as much as possible information from the available reports. Third, I believe that it is important to not only assess the results of formal prospective or retrospective clinical trials, but also observations on "experiments of nature" as well as laboratory data and proxy endpoints that provide supportive evidence that a low estrogen concentration (alone or in combination with a growth promoting agent) is associated with delayed skeletal maturation and increased AH. The complex regulation system of longitudinal growth in the epiphyseal growth plate is influenced by multiple endocrine, paracrine, autocrine and intracrine factors (8), but there is no doubt that the major factor influencing the maturation ("senescence") of the growth plate is the exposure to circulating and intracellular estrogen (9).

Abbreviations: AH, adult height; AIs, aromatase inhibitors; BA, bone age; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; CA, chronological age; CAH, congenital adrenal hyperplasia; CCTs, clinical controlled trials; CDGP, Constitutional delay of growth and puberty; CPP, central precocious puberty; GH, growth hormone; GHD, growth hormone deficiency; GnRH, Gonadotropin Releasing Hormone (GnRH); GnRHa, Gonadotropin Releasing Hormone (GnRH) analogue; HRQoL, Health-related Quality of Life; ISS, idiopathic short stature; NAH, near-adult height; PAH, predicted adult height; LT4, Levothyroxine; RCT, randomized controlled trial; rhGH, recombinant human growth hormone; SDS, standard deviation score; SGA, small for gestational age, also used for a short child born small for gestational age; TH, target height.

Indeed, there are many clinical observations supporting the concept that AH is dependent of the timing and level of circulating estrogens. Untreated children with central precocious puberty (CPP) reach a considerably decreased AH, which can be normalized by timely treatment with a GnRHa (10, 11). At the other end of the spectrum, individuals with decreased circulating estrogens (for example due to hypogonadism) show an extended period of linear growth, resulting in relatively tall stature, even if they are growth hormone deficient (GHD) (12, 13). Proof that estrogen is the primary factor responsible for closure of the epiphyseal growth plates was delivered by the observation that the growth plates of a man with a defect of the gene encoding the estrogen alpha receptor never closed, resulting in tall adult stature (and osteoporosis) (14). Further confirmation was provided by observations that men with aromatase deficiency demonstrated a similar growth pattern, except that growth could be stopped by administering estrogen medication (15). Additional support of the positive effect of postponing exposure to estrogens is provided by the observation that a late start of estrogen substitution in girls with Turner syndrome increases AH (16), although such delay is generally not advised for psychosocial reasons.

The dose-response relationship between estrogen and short term growth, already investigated several decades ago, shows a biphasic curve, with optimal growth occurring at a low estrogen dose (17–20). This was confirmed by the observation that adding low dose estrogen to rhGH treatment increased growth in young girls with Turner syndrome, although the effect was small (21). At the other side of the spectrum, supraphysiological dosages of exogenous estrogens administered to tall female adolescents inhibit growth of the extremities and decrease AH [but please note that this therapeutic approach has become outdated by potential negative long-term effects on fertility (22, 23)]. A consequence of the apparent biphasic dose-response curve is that complete suppression of estrogen exposure may be less beneficial for growth than partial suppression. A recent study showed indeed that a threshold level of estrogen of 10 pmol/L appeared to be needed for an optimal growth rate corresponding to a normal male pubertal growth spurt (24). I shall come back to this issue in paragraph 3, where the use of AIs is discussed.

For each of the referred papers on efficacy reviewed in this paper, I graded the level of evidence (LoE) of studies in a similar fashion as described by the International Consortium on the use of Gonadotropin-Releasing Hormone Analogs in Children (11) (from now on referred to as "International Consortium") using 5 levels: LoE 1 (homogenous randomized controlled trials), 2 (meta-analyses or heterogeneous prospective trials), 3 (case-control studies and retrospective cohorts), 4 (uncontrolled cohort and case studies) or 5 (expert opinions, case reports, and personal observations) (7).

2 THE EFFECT AND SAFETY OF A GnRH ANALOGUE IN CHILDREN WITH A LOW PREDICTED ADULT HEIGHT

On the use of GnRHas in pediatrics, two international conferences have been held. The first was convened in 2007 by

the Pediatric Endocrine Society (PES) from the USA and the European Society for Pediatric Endocrinology (ESPE). The US Public Health grading system (25) was used to grade the evidence and strength of the recommendations, and the participants aimed to adhere to modified appraisal of guidelines research and evaluation (AGREE) criteria (26). The resulting Consensus Statement stated that the efficacy of GnRHas for increasing AH in conditions other than CPP "requires additional investigation and cannot be suggested routinely" (10).

Recently, a second conference was organized by a large number of pediatric endocrine societies (International Consortium) (11), which also included a large section on the use of GnRHas in adolescents with gender dysphoria. The participants aimed to "concisely address topics related to changes in GnRHa usage during childhood and adolescence since the previous consensus statement" and the authors stated that the resulting publication "is not a consensus statement and hence has not been endorsed by any of the societies that designated participating authors." For the purpose of the present review paper, the section on "use of GnRHa in other conditions" is most relevant, and will be discussed in paragraphs 2.2.1 to 2.2.3.

2.1 The Effect of a GnRH Analogue Alone

Treatment with a GnRHa as single treatment is usually effective for children with CPP to reach a normal AH (10, 11). The positive results of GnRHa treatment in CPP triggered several investigators to study the effect of GnRHa on AH in short children with relatively early puberty, albeit still within the population reference range. There are two reports of a small positive effect of a GnRHa in such cases. The only placebo-controlled RCT on the effect of a GnRHa on AH was carried out in 24 children with idiopathic short stature (ISS) and 26 with many different diagnoses. Fourteen of them also received rhGH. The authors reported that GnRHa treatment markedly slowed down further BA progression and significantly increased AH by a mean of 0.6 SD, as compared with PAH at baseline. This effect was independent of sex, the presence or absence of concomitant rhGH, and the presence or absence of a growth-limiting syndrome (27) (LoE 1).

A similar effect was suggested in a recent retrospective cohort study, but only for females (28) (LoE 3). In contrast, four other studies (29–32) (LoE 3) showed no effect. As discussed previously (6), and in line with the International Consortium's opinion (11), my opinion remains that there is insufficient evidence that GnRHa alone increases AH, except for children with CPP.

In order to compensate for the very low height velocity that is often observed during GnRHa treatment in patients with CPP, three approaches have been investigated. First, the combination of GnRHa plus a mini-dose of estrogen was compared with GnRHa alone in patients with CPP in a two year pilot study (33). The results suggested that the low height velocity on GnRHa can be prevented by low-dose estrogen without undue acceleration of bone age (LoE 2), but long-term results have not been reported. Second, the combination of a GnRHa with an anabolic steroid was investigated in two studies with an apparently positive effect on AH compared with matched controls (34, 35) (LoE 3), but since then no more data have been presented. Third, rhGH can be added, which will be discussed in paragraph 2.2.5

There are a number of growth disorders in which predicted adult height (PAH) can be low on conventional disease-specific treatment, which motivated studies on the value of adding a GnRHa. Results of this strategy are discussed in the following paragraphs.

2.1.1 Hypothyroidism

In a retrospective chart review, height outcome and body mass index (BMI) were analyzed in children with severe longstanding hypothyroidism and bone age (BA) delay treated with LT4 alone or with LT4 plus GnRHa (36). Six GnRHa-treated patients and seventeen controls were followed to AH. At diagnosis, GnRHa-treated patients were older and shorter for chronological age, and more advanced in puberty and BA compared to controls on LT4 alone. Despite these baseline differences (which would predict a lower AH for the experimental group), both groups showed similar improvements in height standard deviation score (SDS), similar height deficits and comparable AH, which was interpreted as a positive therapeutic effect (LoE 3). Changes in BMI SDS were similar for both groups (36).

2.1.2 Laron Syndrome

Children with GH insensitivity syndrome (Laron syndrome) treated with recombinant human IGF-I show a modest growth acceleration, but AH is usually still below the population range. A few reports [e.g (37, 38)] suggested that adding a GnRHa may increase AH if started at the onset of puberty (LoE 4), but controlled studies have not been reported.

2.2 The Effect of a GnRH Analogue in Combination With Recombinant Human Growth Hormone

Information on efficacy and safety of GnRHa plus rhGH is available for the following conditions: 1) growth hormone deficiency (GHD); 2) short stature after being born small for gestational age (SGA); 3) ISS; 4) haploinsufficiency of *SHOX*, *NPR2* or *ACAN*; 5) CPP; 6) congenital adrenal hyperplasia (CAH); and 7) hypothyroidism. For the first two conditions and *SHOX* haploinsufficiency rhGH treatment is approved in most parts of the world, and for ISS it is registered in the USA and some other countries. For all other conditions reviewed here, the use of rhGH is off label. GnRHa treatment is off-label for all pediatric conditions except CPP.

2.2.1 GH-Deficient Children

The most logical group of GH deficient children who may benefit from the addition of a GnRHa are children who develop CPP before or during rhGH treatment, for example children who had undergone treatment of malignancies (39–42) (LoE 2). The recent conference report stated that adding a GnRHa leads to increased PAH and AH in such cases (11).

A second class of GH deficient children in whom co-medication with a GnRHa can be considered are those who have not reached full catch-up growth at the onset of puberty (11). Longitudinal data on height in rhGH-treated children with

GHD have shown that height SDS at the onset of puberty is similar to AH SDS (43, 44). This implies that a low height SDS at pubertal onset, even if this is normally timed, likely leads to a low AH SDS. This was the reason that a prospective RCT was carried out in Santiago (Chile) in collaboration with the National Institutes of Health (Bethesda, USA) to compare near-adult height (NAH) of treatment-naïve GHD patients (in Tanner stage II-III, females premenarcheal) treated with either rhGH plus GnRHa (experimental group, n=7) or rhGH alone (controls, n=10) (45). rhGH was administered until patients reached NAH, and the GnRHa was given for 3 years. BA advancement was significantly different between groups in the 3 year interval (1.5 ± 0.2 "years" on rhGH plus GnRHa versus 4.2 ± 0.5 on rhGH alone), and NAH was-1.3 ± 0.5 versus -2.7 ± 0.3 SDS, respectively (LoE 1). The difference in NAH was close to 10 cm. While I agree with the authors' conclusion that these results indicate that delaying puberty with a GnRHa in GHD children during treatment with rhGH increases AH, I wish to call the readers' attention to the fact that the patients in this study were quite different from the usual GHD patient in countries where rhGH is approved and reimbursed for this indication. These severely GH-deficient patients entered into the study at a remarkably late mean age of 14.3 years, BA of 11.3 years, height of -4.3 SDS, and PAH of -3.1 SDS. They are therefore not representative for the majority of GHD patients in whom rhGH is typically started within the first decade of life with less severe growth delay.

One year later our group reported on a retrospective analysis of the effect of the addition of GnRHa (started shortly after the onset of puberty) to rhGH treatment in children with GHD and a low height SDS [mean (SD) -3.0 (1.5)] at onset of puberty (43). Matched controls with rhGH treatment only were used for comparison. The children were younger (mean age 8.9 years), equally short at start of rhGH treatment, but less short at the start of GnRHa compared with the subjects in the Chilean RCT (45). The effect of GnRHa addition on AH [in terms of AH minus target height (TH) SDS] was estimated at 1.2 SDS (approximately 8.5 cm) (LoE 3), so similar to the results of the RCT. We later described an even more impressive height gain of 25-30 cm as a result of the addition of a GnRHa in two siblings with severe GHD due to a homozygous *GHRHR* defect who moved to the Netherlands at mid-puberty and started rhGH plus GnRHa treatment at that time (46) (LoE 4).

In the International Consortium's report it was concluded that "the addition of a GnRHa to GH at the onset of puberty and treatment for at least 2 years resulted in gains of AH ranging from 6 to 9 cm (∼1–1.5 SD)", in line with my conclusion. This observation is also compatible with the observations in patients with undiagnosed or untreated combined deficiency of GH and gonadotropic hormones, who can reach a normal height or even turn "from dwarfs to giants" (12, 13).

Regarding safety of this approach, there is a theoretical risk of decreased bone mineral content (BMC) of 2-3 years of GnRHa. This was investigated in the Chilean RCT (45) and in fact patients treated with rhGH plus GnRHa had a significantly lower BMC after 3 years of therapy. This difference, however, did not persist after both groups of patients reached NAH (47). Another potential adverse event is disproportionate growth (potentially leading to a eunuchoid shape), but this was not observed in the RCT (45). The psychological profiles of the patients who participated in this study showed that their first priority was to increase linear growth, whereas pubertal progression was felt to be of lesser importance, particularly for boys (45).

2.2.2 Short Children Born Small for Gestational Age

Pubertal height gain is often less than expected in children born SGA, as a result of an earlier onset of puberty, an earlier peak height velocity, and accelerated bone maturation (11, 48, 49). Therefore, theoretically, it would make sense to add a GnRHa if height SDS is low at pubertal onset in order to increase AH. The results of three studies are in favor of this hypothesis [reviewed in (50)].

First, in a clinical trial on rhGH-treated short early pubertal children born SGA, patients were randomized into 2 groups (rhGH dosage of 1 or 2 mg/m^2.day). Children with a height below 140 cm (-2.5 SDS in the Netherlands) at pubertal onset received GnRHa co-treatment for 2 years, and their growth was compared with that of children with a height above 140 cm at pubertal onset receiving rhGH only. In children treated with rhGH plus GnRHa, the total height gain on GH treatment in both dosage groups (34.5 cm and girls 24.2 cm for boys and girls, respectively) was larger than expected for the general population, in spite of a shorter pubertal duration after discontinuation of GnRHa (51, 52) (LoE 2). As a result, AH of these rhGH plus GnRHa treated children was similar to AH of rhGH-treated children who were >140 cm at pubertal onset, which would not have been expected without GnRHa co-medication. Extensive studies on this cohort during and up to 5 years after cessation of rhGH regarding body composition, metabolic profile, bone health, cognition, self-perception, behavior and Health-related Quality of Life (HRQoL) showed no adverse effects of the addition of the GnRH analogue (53–57). It is noteworthy that the initially proposed research design (an RCT on the addition of GnRHa) was turned down by the medical ethics committee, because it was expected that adding a GnRHa would have a positive effect on AH.

Second, the combination of rhGH plus GnRHa versus untreated controls for 3 years in short children born SGA or with a normal birth weight (idiopathic short stature, ISS) showed a positive effect on AH in an RCT, but only in girls (58) (LoE 1). Third, a French retrospective study (59) showed that after 4.6 ± 2.8 years of rhGH treatment, height SDS of short children born SGA increased from -2.2 ± 0.9 to -1.5 ± 0.9, and that in a multivariate analysis, the use of a GnRHa therapy for at least 2 years was one of the eight predictive factors (estimated effect 0.4 SD) (59) (LoE 3). The only report suggesting that adding a GnRHa to rhGH treatment had no positive effect on AH was based on a retrospective analysis (60) of non-standardized GnRHa treatment in 16 of 37 patients with Silver-Russell syndrome (LoE 4) (61).

My interpretation of the available data goes a step further than the one taken by the International Consortium ("it is appropriate to consider the potential advantages and disadvantages of treatment with GH and GnRHa in this

population") and contrasts with a recent opposite view (62). I believe that there is sufficient evidence that co-treatment with a GnRHa for 2-3 years does increase AH in rhGH treated short SGA-born children if height SDS is <-2.5 at pubertal onset.

2.2.3 Idiopathic Short Stature

Unlike the previous two conditions, ISS is not an approved indication for rhGH in most parts of the world (except the USA and a few other countries), although reports on post-marketing databases suggest that it has been widely prescribed off label in other countries as well (63). Several studies have been performed on the potential efficacy of the combination of rhGH plus GnRHa in ISS, which can be divided into five sets.

First, there are four reports without follow-up till AH, in which the change in PAH was used as outcome marker for efficacy (64–67). These reports will not further be discussed due to the unavailability of attained AH. The second set contains two uncontrolled studies (68, 69). The third set comprises two controlled studies on a short course of rhGH plus GnRHa (58, 70). Set 4 contains two studies comparing the effect of the combination treatment with that of rhGH alone (71, 72). Set 5 is the retrospective Israeli study in prepubertal and pubertal children on the effect of a GnRHa in addition to long-term rhGH treatment (73). Relevant clinical data from the studies in sets 2-5 are presented in **Table 1**.

The two uncontrolled studies in set 2 (LoE 4) presented contradictory results. The Italian study (68) showed no positive effect, while the authors of the Chinese study (69) concluded that GnRHa/rhGH therapy can effectively improve AH SDS up to TH SDS.

Similarly, the results of the two studies in set 3 were contradictory. The small prospective Venezuelan study compared the growth response to rhGH plus GnRHa with that of untreated historical controls (LoE 3) and reported no difference (70). In contrast, the RCT of our group in short children born with a low or normal birth size (58) (LoE 1) showed a mean positive effect on AH (5 cm), with a clear difference between sexes: mean (SD) AH-TH was -3.3 (5.9) cm in the treated group versus -12.0 (5.3) cm in untreated controls ($p<0.05$) in girls, compared with -11.8 (6.5) versus -9.7 (6.9) cm in boys (NS). It is noteworthy that of the PAH increase of 9.3 cm between start and discontinuation of treatment, almost 50% was "lost" between the end of medication and AH. In this study, no long-term negative or positive psychosocial consequences were observed (74), bone mineral density (BMD) did not change significantly, and the effect appeared similar for ISS and SGA (58).

TABLE 1 | Results of studies on the efficacy of rhGH plus GnRHa on adult height in children with idiopathic short stature.

Design	Uncontrolled studies		Short combo vs no treatment		Combo vs rhGH alone		Adding GnRHa to rhGH treatment	
Ref	(68)	(69)	(70)	(58)	(71)	(72)	(73)	
Diagnosis	ISS	ISS	ISS	ISS+SGA (RCT)	ISS	ISS (RCTdiscont[1])	ISS prepub	ISS pub
Duration rhGH, y	2.3	2	2.5	3	4.6	2.4	7.8 vs 7.0M, 5.9 vs 5.6F	4.2 vs 3.1M, 3.7 vs 2.9F
GnRHa, y	2.3	2	2.5	3	4.6	2.4	2.0M, 1.8F	2.0M, 1.8F
Controls	–	–	Hist, No R/	No R/	Hist, rhGH 4.9y	rhGH, 2.4y	rhGH	rhGH
N, sex	10F	25M, 12F	10 vs 10 3M, 7F	17 vs 15 11M, 21F	12 vs 12 F	19 vs 23M 26 vs 20F	12 vs 62M 19 vs 33F	8 vs 28M 19 vs 11F
Age, y	11.6	13.8M 12.6F	11.8 vs 11.4	11.6 vs 11.8	10.2 vs 10.7	12.1 vs 12.1	8.3 vs 9.4M 9.1 vs 9.0F	12.9 vs 14.3M 11.3 vs 12.7F
HSDS0	-2.7	-2.8M -3.1F	-2.4 vs -2.3	-2.4 vs -2.5	–	-2.5 vs -2.5	-2.4 vs -2.6M -2.7 vs -2.7F	-2.3 vs -2.7M -2.1 vs -2.7F
ΔPAH	3.0 (1y) cm		0.7 vs -0.6 cm	9.3 vs 1.2 cm	10.5 vs 7.9 cm			
AH	-2.8 SDS	-1.8M -0.3F	151.7 vs 150.3 cm	-2.0 vs-2.3 SDS	156.3 vs 146.3 cm	-1.9 vs -1.8 SDS[1]	-0.5 vs -0.8M -0.7 vs -0.7F SDS	-0.5 vs -0.7M -1.1 vs -1.1F
AH-HSDS0	-0.1	2.0M 2.7F		0.5 vs 0.3	–		1.9 vs 1.8M 2.0 vs 1.9F	1.8 vs 2.0M 1.6 vs 1.1F
AH-PAH, cm	1.4	5.3M 12.8F	1.0 vs -1.5	4.4 vs -0.5	10.0 vs 6.1		8.4 vs 6.3M 11.9 vs 12.7F	7.6 vs 4.7M 9.5 vs 7.2F
AH-TH	-3.0 cm	-0.1 SDS M 0.5 SDS F	-10.0 vs -9.6 cm	-6.0 vs -11.2 cm	3.6 vs -4.1 cm		0.6 vs 0.3M 0.2 vs 0.1F SDS	0.1 vs 0.1M 0.3 vs -0.0F SDS
Total pubertal height gain, cm							34.4 vs 26.8M 27.5 vs 18.7F	35.8 vs 29.0M 25.7 vs 21.9F

[1]*Trial was abrupted after 2.4 years, thus severe loss to follow-up. NAH was reported on a small sample only.*

AH, adult height; Combo, combination; discont, discontinued; F, females; HSDS0, height SDS at start of treatment; hist, historical; ISS, idiopathic short stature; M, males; PAH, predicted adult height; R/, treatment; RCT, randomized controlled trial; SGA, small for gestational age; TH, target height; vs, versus; y, year.

In set 4, the effect of rhGH plus GnRHa for a relatively long period (4.6 years) was compared with that of rhGH-treated matched controls in girls (71). The result was impressive (AH 156.3 ± 5.9 vs. 146.3 ± 5.0 cm, so 10 cm difference). The large RCT comparing the combination treatment with rhGH alone (72) was unfortunately aborted at the request of the French regulatory authorities (LoE 2). NAH was only reported on 35 of the included 91 children, which did not show a difference between treatment regimens. Bone fractures occurred more frequently in the GH plus leuprorelin group than in the GH alone group (seven versus 3, respectively), but no bone fractures were reported during the safety follow-up period.

Finally (set 5), the effect of adding GnRHa in puberty was investigated in a retrospective analysis on 58 out of 192 children who either started rhGH treatment before (n=31) or during puberty (n=27) (73). The authors concluded that combined rhGH/GnRHa therapy increased AH outcome, and that this effect was more pronounced in the prepubertal group and in girls. The mean effect on total pubertal growth was 8-9 cm (LoE 3).

Though strictly speaking short, adopted girls should not be labeled ISS, I also mention in this paragraph two studies on adopted girls showing an estimated mean height gain of 3-4 cm on rhGH plus GnRHa versus GnRHa alone (LoE 1) (75, 76).

The general picture arising from these studies is that in countries where rhGH can be prescribed for children with ISS, clinicians can consider adding a GnRHa if puberty starts when height SDS is still below the reference range at onset of puberty, particularly in girls (58, 71, 73). A longer duration of GnRHa co-medication than 3 years may have a larger effect on AH (71), but its potentially negative psychosocial consequences should be considered as well.

2.2.4 Haploinsufficiency of *SHOX*, *NPR2*, or *ACAN*

In children with haploinsufficiency of *SHOX*, *NPR2* or *ACAN*, the prepubertal growth rate is relatively well-preserved but followed by a compromised pubertal growth spurt due to premature growth plate fusion, so that height SDS decreases by age (77–79). *SHOX* haploinsufficiency is a registered indication for rhGH since 2006 (80), but this does not apply to the two other genetic syndromes. The few anecdotal reports on the administration of GnRHas as co-treatment to rhGH in children with these genetic defects are reviewed in the next paragraphs.

2.2.4.1 SHOX Haploinsufficiency

In a retrospective analysis of 10 children with *SHOX* haploinsufficiency (77), five patients were followed without treatment, and five were treated with rhGH (50 μg/kg.d) plus GnRHa. Mean AH SDS minus height SDS at first evaluation was significantly different between treated (+0.6) versus nontreated (-1.2) patients (77) (LoE 4), but obviously the relative contribution of the two components of this combined therapy is unknown. In a Japanese paper two girls with *SHOX* haploinsufficiency were treated with rhGH and a few years of GnRHa, but the reported data do not allow for assessing the contribution of GnRHa co-treatment (81) (LoE 4).

2.2.4.2 NPR2 Haploinsufficiency

In a recent paper the data of 21 patients with heterozygous *NPR2* variants who were treated with rhGH were summarized. In three of them a GnRHa was added and in one case letrozole (82). The first reported case (83) was treated with a GnRHa for 1.5 years from 13.3 years onward and with rhGH (33 μg/kg.d) for 3.3 years from the age of 13.8 years. Height SDS increased from -2.8 to -2.5 SDS and AH was 158 cm, similar to the heights of his affected father and grandfather, suggesting no beneficial effect (LoE 4).

In the same paper (83), the results were reported of treatment of rhGH (50 μg/kg.d) plus GnRHa in a 12.8 year old boy. Puberty had started at 12.1 years, when he was 131 cm and had a PAH of 160 cm. After 2.7 years of treatment, GnRHa was discontinued. He reached an AH of 164 cm, suggestive for an AH gain of 4 cm (LoE 4).

Growth data of the third case (84), showed that treatment with rhGH for 11.6 years and an unreported duration of GnRHa led to a height SDS increase from -3.8 to -3.1 SDS (82) (LoE 4). Taken together, rhGH plus GnRHa appears to have a modest AH-augmenting effect, but the respective roles of rhGH and GnRHa cannot be assessed in this condition.

2.2.4.3 ACAN Haploinsufficiency

Approximately 50% of children with *ACAN* haploinsufficiency present with an accelerated BA (85) and mean height SDS decreases by age (79), so clinicians have been tempted to investigate the potential role of rhGH plus GnRHa to increase AH. In a large international patient series, five children received rhGH plus GnRHa, which indeed appeared to halt skeletal maturation (79) (LoE 4). In a recent report on 6 novel cases with heterozygous *ACAN* variants, the authors reviewed previously published cases who were treated with rhGH with (n=11) and without (n=10) a GnRHa or an AI (86) (LoE 4). Unfortunately, no separate analysis was made for GnRHs and AIs. No statistically significant differences were found in terms of AH, but the presumably wide interindividual differences in age at start of medication and other relevant clinical variables make it difficult to interpret these findings. Specifically in patients with *ACAN* haploinsufficiency, accelerated epiphyseal fusion during pubertal years remains a worrisome clinical problem and studies investigating add-on GnRHa or AI therapy in these patients should be a priority.

2.2.5 Central Precocious Puberty Associated With a Persistently Low Predicted Adult Height

While in most cases of CPP treatment with a GnRHa leads to an increase of PAH and a normal AH (10), there are patients with CPP in whom PAH remains low on GnRHa alone, usually associated with a very low height velocity. The addition of rhGH to GnRHa treatment in 10 girls for 2-3 years led to an AH gain of 7.9 cm in comparison to 1.6 cm with GnRHa alone in matched controls (87) (LoE 3). Longer follow-up in a larger cohort showed a mean additional AH gain of 6 cm (88) (LoE 3). Of note, rhGH has not been registered for this indication by any of the drug regulatory agencies.

An observational study on rhGH plus GnRHa showed that this treatment was associated with a height gain of 5.4 cm in 18

girls with CPP, compared to 3.0 cm in 62 girls receiving the same treatment but with normal onset of puberty (42) (LoE 4).

A meta-analysis of controlled studies in CPP with severely decreased growth velocity during GnRHa therapy (89) reported an increased AH in patients with GnRHa plus rhGH versus GnRHa alone [+2.8 cm in four clinical controlled trials (CCTs) and +4.3 cm in one RCT], as well as greater AH-TH (+3.9 cm in the CCTs and +4.0 cm in the RCT) and greater AH-PAH (+3.5 cm in the CCTs and +3.9 cm in the RCT). Patients with a low growth velocity or no improvement in PAH during GnRHa benefitted most from the combination therapy (89) (LoE 2). A systematic review and meta-analysis reported an increase of PAH of 6.5 cm in CPP when treated with rhGH plus GnRHa, especially in those starting treatment before 10 years old, or with treatment lasting more than 12 months (90). Compared to GnRHa alone, the combined treatment showed a 3.7 cm higher PAH (LoE2). AH was not reported.

In a large retrospective analysis on girls with CPP or early and rapidly progressive puberty (91), the effect on AH of GnRHa plus rhGH treatment, GnRHa alone, or no treatment were compared. Compared to no treatment, GnRHa alone yielded an AH gain of 1.5 cm and when combined with rhGH an additional 1.5 cm was gained. Compared to TH, GnRHa alone showed an increase of 2.0 cm and combined with rhGH a total increase of 4.0 cm, while controls reached their average TH. An AH-PAH of at least +5 cm was reached in 60% of controls, 70% of GnRHa and 75% of those with combination therapy, especially those with a more advanced BA and low PAH. Lower percentages were seen when the TH-AH endpoint was used (10%, 25% and 45%, respectively) (LoE 3).

2.2.6 Congenital Adrenal Hyperplasia

In general, growth of children treated for CAH stays within the population range if glucocorticoid dosage is minimized (to avoid iatrogenic Cushing's syndrome), mineralocorticoids are properly dosed and supplemental sodium is given to infants (92). The Endocrine Society Clinical Practice Guideline recommended against routine use of experimental therapies to promote growth and delay puberty (92). However, several retrospective studies showed that mean AH of children treated for CAH is approximately 1 SD below the population mean, probably due to a combination of the condition itself and its treatment (93).

Several groups have tried to increase AH by adding rhGH plus GnRHa (both off-label for this indication) to the conventional treatment with hydrocortisone and fludrocortisone acetate. In a study comparing 2 years of rhGH alone or rhGH plus GnRHa co-treatment versus controls without such treatment, mean PAH increased by 11 cm in both experimental groups combined and closely approximated TH, while PAH did not change in controls. There was no difference between the groups treated with rhGH alone and those treated with the combination of rhGH with GnRHa (94) (LoE 3).

In another study, rhGH plus GnRHa therapy was given for 4.2-4.4 years and results were compared with matched controls. In the treatment group, AH SDS was 1 SD greater than both the initial PAH SDS and AH SDS of the untreated group (95) (LoE 3). Six years later, in a nonrandomized prospective study, the same group reported 34 patients that were predicted to be more than 2 SD below TH or the population mean at around 8 years old, treated with either rhGH alone (n=7) or combined with GnRHa in case of precocious or early puberty (n=27). AH was 9.2 ± 6.7 cm higher than initial PAH in males, and 10.5 ± 3.7 cm in females (96) (LoE 3). There were no differences between the two treatment options, although the rhGH alone group started treatment at a later age. In subjects with poor adrenal control (all males), the gain was only 4.0 ± 3.0 cm.

In a retrospective analysis (97), 13 patients were treated with rhGH plus GnRHa. rhGH was given for 1.0-6.3 years and GnRHa for 2.1.-6.2 years. On average, an increase of 2 SD in height SDS for BA was noted in the first years after treatment, which remained stable until NAH was reached (LoE 3). In another study (98), 32 CAH patients with CPP were treated with GnRHa (n=11), GnRHa plus letrozole (n=11), or no additional treatment (n=10). Compared to no additional treatment, only the GnRHa plus letrozole group had a higher NAH (-1.3 vs -2.5 SDS) (LoE 3). A Chinese retrospective study compared the effect of two regimens: rhGH plus GnRHa and rhGH plus GnRHa plus letrozole (for an average period of 25 months). PAH increased by 9 cm versus 12 cm, respectively (99) (LoE 3).

From the available data I conclude that in the subset of CAH patients with a low PAH at pubertal onset, adding rhGH alone or in combination with a GnRHa may increase AH.

2.2.7 Hypothyroidism

In a case-report two patients with severe acquired juvenile hypothyroidism presenting with compromised PAH were treated with rhGH plus GnRHa in addition to LT4 (100). In the first patient, a 13 year-old girl, PAH decreased to 144 cm after one year of LT4 treatment. rhGH plus GnRHa for one year slowed BA progression, and led to an AH of 155 cm (LoE 4). In the second patient, a 14 year-old boy, a 2 year treatment with rhGH plus GnRHa was initiated in addition to LT4 leading to improvement of growth velocity (10.6 cm/yr) while slowing bone age progression, resulting in an AH equal to TH, an increase of 10 cm compared with PAH after one year of LT4 treatment (LoE 4). When one compares these observations with the study on the effect of the addition of GnRHa alone (36) (paragraph 2.1.1), the addition of rhGH appears crucial for a substantial height gain, but obviously observations in two patients are insufficient for a reliable assessment.

2.3 Safety of GnRHas in Childhood and Adolescence

According to the GnRHa consensus meeting (10), "GnRHas are generally well tolerated in children and adolescents. Systemic complaints such as headaches or hot flashes occur occasionally but are usually short-term and do not interfere with therapy. Local adverse events occur in 10-15% patients and necessitate a change in agent when persistent, because they can result in sterile abscesses in a fraction of the patients. Although exceedingly rare, anaphylaxis has been described". The International Consortium gave a very elaborate description of possible adverse events, but basically the conclusion was the same: "Adverse effects of GnRHa

therapy are rare, and the associations of most reported adverse events with the GnRHa molecule itself are unclear. Decades of experience have shown that GnRHa treatment is both safe and efficacious" (11). In a few studies negative effects on bone acquisition have been reported (27, 45, 58), but these appear transient (47).

The main downside of GnRHa treatment is that it brings the early- or mid-pubertal adolescent back to a prepubertal state, not suitable to his or her age, which may lead to psychosocial issues and differences in behavior and interests compared with peers.

3 THE EFFECT OF AROMATASE INHIBITORS

While GnRHas can be considered as having a "semi-physiologic" effect (by bringing the body back to a prepubertal state), AIs are clearly pharmacological agents. They are registered for the treatment of estrogen-dependent breast cancer, and aimed at decreasing the exposure to estrogens as much as possible, primarily by inhibiting the intracellular conversion of androgens to estrogens, but also by decreasing circulating estrogen concentrations. At present, there are three so-called third generation AI compounds registered: letrozole (2.5 mg o.d.), anastrozole (1 mg o.d.) and exemestane (25 mg o.d.). The effect of letrozole on aromatase inhibition is stronger compared to that of anastrozole [88% vs 85% tissue aromatase blockade in postmenopausal women (101)], with mean residual estradiol concentrations of 10.1% for anastrozole and 5.9% for letrozole (102), consistent with observations in boys with ISS (103). Exemestane is a nonsteroidal aromatase inhibitor, and covalent binding of the drug to the active site of the enzyme irreversibly inhibits aromatase action, in contrast to the two non-steroidal AIs which form a reversible bond with the enzyme [reviewed in (5)].

AIs are not registered for any pediatric indication and as far as I know no basic pharmacological studies have been performed in children or adolescents. Therefore, no information is available on the optimal dosages for the three compounds if they would be used in childhood and adolescence. In fact, in pediatric studies the same dosage has been used as for adults, which probably leads to maximum suppression of aromatase. The use of AIs has been investigated in four groups of conditions: hyperestrogenism, hyperandrogenism, pubertal gynecomastia, and short stature and/or delayed puberty (5).

3.1 Use of Aromatase Inhibitors in Hyperestrogenism, Hyperandrogenism, or Pubertal Gynecomastia

Four rare disorders characterized by hyperestrogenism (aromatase excess syndrome, Peutz-Jeghers syndrome, McCune-Albright syndrome, and functional follicular ovarian cysts) are logical indications for AIs. For details I refer to a previous review (5) and a recent paper on Peutz-Jeghers syndrome (104).

Regarding hyperandrogenism, the efficacy of AIs in testotoxicosis, also known as familial male-limited precocious puberty, is well established (5). The positive effect of long-term treatment with the combination of an antiandrogen, aromatase inhibitor and GnRH analogue was recently confirmed (105, 106) (LoE 2).

As mentioned in paragraph 2.2.6, the conventional treatment of children with 21-hydroxylase deficiency with a combination of hydrocortisone and fludrocortisone acetate may in some children lead to a low AH. A lower dosage of corticosteroid treatment in combination with a first generation AI and an androgen antagonist appeared efficacious (107), but became outdated by the arrival of second and third generation AIs.

The addition of letrozole to CAH patients with concomitant CPP who received a GnRHa led to a significantly higher NAH compared with the no intervention group (98) (LoE 3). In a recent case report the effect of 9 years of exemestane, combined with 4 years of GnRHa treatment because of early central puberty, was described in a patient with markedly advanced BA (108) (LoE 4). At start of exemestane the PAH was -4.6 SDS and at its discontinuation NAH was -0.8 SDS. In another study, already mentioned in paragraph 2.2.6, the addition of letrozole to rhGH plus GnRHa had an additional effect of 3 cm (99) (LoE 4).

Also patients with an 11 β-hydroxylase deficiency can end up short, particularly if the diagnosis is made during later childhood. The first report on 11 years of administration of letrozole without rhGH in a boy with 11β-hydroxylase deficiency demonstrated a 35 cm increase in AH over PAH (109) (LoE 4). This was combined with a GnRH analogue for 2.5 years due to central activation of puberty. However, significant adverse events were reported in this patient: back pain and vertebral changes were noted from the age of 15 years onwards, as well as impaired sperm motility and subnormal morphology. Two case reports showed that the addition of rhGH and an AI to glucocorticoid replacement was highly efficacious (LoE 4). The first case (110) was treated with rhGH and letrozole resulting in a higher AH as compared with PAH. The second case (111), treated with rhGH plus anastrozole, reached an AH of 11.5 cm above TH. In these patients no side effects were noted.

While initially AI treatment appeared to be a rational approach in boys with pubertal gynecomastia, the results have been disappointing (5). In a recent clinical practice guideline the use of selective estrogen receptor modulators, aromatase inhibitors, or non-aromatizable androgens was not recommended for this self-limiting condition (112).

3.2 Use of Aromatase Inhibitors in Boys With Short Stature and/or Delayed Puberty

Third generation AIs have been used as a potential agent to enhance AH, particularly letrozole (2.5 mg o.d.) and anastrozole (1 mg o.d.). The motivation to initiate such studies was the observation that males with a pathogenic variant of the genes encoding the estrogen receptor or aromatase show a substantial BA retardation while their stature in childhood and adolescence was normal, resulting in increased AH (14, 15, 113–116).

Regarding the use of AIs in children, there is one systematic review by the Cochrane group (117), which concluded that the "available evidence suggested that aromatase inhibitors improved short-term growth outcomes", but that "there was no evidence to support an increase in AH, based on limited data, with only one of four trials publishing AH data under non-randomized conditions" (LoE 2).

In most reports and reviews on AIs, the authors apparently assumed that the effects of letrozole and anastrozole are similar regarding growth and skeletal maturation. In contrast, I believe that there is circumstantial evidence that the effects differ to some extent, which may well be related to the different degree of blocking aromatase. The biphasic dose-response relationship of estrogens and growth discussed in paragraph 1 would suggest that a total blockade of estrogen exposure may have a negative effect on growth, which could serve as an argument in favor of anastrozole above letrozole.

Unfortunately, there is little information about a direct comparison between the efficacy and safety of both compounds in childhood. There is only one study in which the effect was studied on growth and BA in short pubertal males. First year height velocities were similar, but PAH increased more in the anastrozole group (118) (LoE 3). The study in which either letrozole or anastrozole was administered to boys with ISS (103) was not powered to detect differences between the effect of these drugs on growth and skeletal maturation. Similarly, there is no direct comparative information about adverse events in adolescents of both compounds, but the published data seem to suggest that adverse events are seen more frequently on letrozole treatment than on anastrozole.

In the following paragraphs I summarize the outcome of clinical studies in children/adolescents at risk of a low AH, as a follow-up to several previous reviews (1, 3, 5, 119–122).

3.2.1 Idiopathic Short Stature, Growth Hormone Deficiency, and Constitutional Delay of Growth and Puberty

Several studies have been performed with AIs in boys with ISS, constitutional delay of growth and puberty (CDGP) (which I consider equivalent to ISS with delayed onset of puberty) or GHD (5). Auxological data from several studies are presented in **Tables 2**, **3**. Given the possible differences in efficacy and safety between letrozole and anastrozole, the results will be discussed in separate paragraphs for studies on letrozole, anastrozole and studies in which both were used.

3.2.1.1 Letrozole

The first RCT on the effect of letrozole in boys with CDGP (128, 129) appeared to lead to a small gain of NAH (LoE 1), but as discussed previously (5) there are several issues which make this claim uncertain. The most important issue is potential selection bias at start of treatment and in the final analysis. Further, results obtained with a combination treatment (letrozole plus testosterone) cannot be extrapolated to letrozole alone. In addition, no AH data were reported for the untreated boys, and the attained AH might be considerably higher than the NAH, since the range of bone ages at follow-up was quite wide (15.8–18.0 years).

The same Finnish group also performed an RCT on the effect of a 2 year course of letrozole vs. placebo in peripubertal boys with ISS (prepubertal in 90%) (**Table 2**). At discontinuation of medication there was a 5.9 cm increase of PAH on letrozole (123), but the authors have to be praised for performing a study on attained AH. This showed that there was no statistically significant difference in AH (124) (LoE 1). Of note, in both groups, the attained AH was 3 cm lower than PAH at start of treatment (124). Regarding the study design, in hindsight, it would have been more logical to start letrozole in early- or midpuberty than before puberty. Minor vertebral deformities occurred in boys who were prepubertal at start (130), but this ameliorated with time (124). The results of this study serve as a warning against overestimating the accuracy of PAH calculations after a therapeutic course with a puberty modulator.

In an Iranian study, 91 boys with CDGP were randomly allocated to letrozole, placebo or oxandrolone for 2 years (131). The results showed several questionable issues (5) and unfortunately we have been unable to obtain clarifications from the authors (LoE 4). In another Iranian study (132) the effect of one year on letrozole versus no treatment in small groups of 6 boys was reported. The authors concluded that letrozole treatment was associated with an AH of 1.9 cm above PAH, in contrast to no increase in controls (0.1 cm). However, the letrozole group was investigated at a considerably older age than the controls (23.4 versus 19.9 years) (LoE 3).

In a retrospective chart review of 21 boys with predicted short stature and/or rapidly advancing BA, due to many different diagnoses, 19 received letrozole, one subject anastrozole, and one subject initially anastrozole for 1 year followed by a switch to letrozole. No increase of PAH was observed, regardless of Tanner stage (2) (LoE 4).

Finally, a recent Finnish randomized controlled phase 3 trial tested whether letrozole for 6 months might be a feasible alternative treatment to low-dose testosterone for boys with CDGP (133). Thus, this study was not aimed at investigating whether letrozole would result in a taller AH. In the letrozole group serum concentrations of LH, FSH, testosterone and inhibin-B as well as testicular volume were higher than in the testosterone group, but height velocity was slightly lower (LoE 1). The safety profile of both regimens was satisfactory, but the authors warned that "the risks and benefits of manipulating the reproductive axis during early puberty should be weighed carefully" (133).

A case report of a GH deficient boy who received letrozole for 17 month in addition to rhGH suggested a positive effect on PAH, but unfortunately no data on attained AH were reported (134) (LoE 4). Another case report on the effect of 5 years of letrozole in a 14.5-year-old boy with ISS demonstrated that AH surpassed the pre-treatment PAH by 15 cm (135) (LoE 4).

3.2.1.2 Anastrozole

In an RCT from the USA, 52 adolescent males with GH deficiency treated with rhGH were randomized to cotreatment with anastrozole or placebo daily for up to 36 months. Anastrozole cotreatment was associated with slower bone maturation and higher PAH while maintaining normal pubertal progression after 2–3 yr (125) (LoE 1) (**Table 3**).

TABLE 2 | Effect of letrozole (Let) or anastrozole (Ana) on growth in boys with idiopathic short stature (ISS).

Country, Diagnosis (duration)	Finland, ISS (2 yrs)		US, ISS, 1 yr		US, ISS, 2 yrs		
Reference	(123, 124)		(118)		(103)*		
Medication	Let	Placebo	Let	Ana	Let/Ana	GH	Let/Ana+GH
N	10	10	17	22	25	25	26
At start							
Age, yrs	11.5 (1.8)	10.9 (1.8)	14.1 (1.3)	14.1 (1.4)	14.2 (0.2	14.1 (0.2)	14.0 (0.2)
Hgt, cm	129.7 (7.9)	127.5 (7.5)	148.7 (6.2)	149.3 (6.7)	145.7 (1.1)	144.2 (1.4)	144.5 (1.3)
Hgt SDS	-2.4 (0.3)	-2.5 (0.4)			-2.2 (0.1)	-2.4 (0.1)	-2.3 (0.1)
HV, cm/yr			7.1 (3.0)	6.0 (3.5)			
TH, cm			175.3 (4.5)	173.8 (8.7)	171.8 (0.8)	170.1 (1.3)	171.6 (0.9)
BA, yrs	9.2 (2.6)	8.7 (1.9)	13.3 (0.7)	13.4 (0.8)	12.8 (0.3)	12.9 (0.3)	12.7 (0.2)
PAH, cm	167.6 (7.9)	166.9 (3.9)	166.4 (4.5)	165.7 (5.2)			
Testic vol, ml	1.5 (1.4)	1.0 (0.6)	8.3 (3.2)	7.7 (3.5)			
Tanner G	8/2/0/0/0	10/0/0/0/0	0/8/8/1/0	0/9/9/4/0	2-3	2-3	2-3
T, nmol/L	1.4 (1.9)	0.4 (0.4)			7.1 (1.3)	8.5 (1.4)	7.7 (1.3)
At stop							
Age			15.2 (1.3)	15.2 (1.5)			
Hgt, cm			156.4 (5.1)	157.6 (6.7)			
Hgt SDS					-1.73 (0.1)	-1.43 (0.1)	-1.25 (0.1)
HV, cm/yr			7.2 (2.1)	7.2 (1.8)			
Δ hgt,cm					14. (0.8)	17.1 (0.9)	18.9 (0.8)
BA, yrs	10.2 (2.9)	10.8 (1.5)	14.2 (0.8)	14.2 (0.9)			
ΔBA, yrs	1.24#	2.05#			2.1 (0.3)	2.5 (0.1)	1.9 (0.2)
PAH, cm	174.0 (8.3)	167.4 (4.3)	167.7 (5.6)	169.9 (6.3)			
HSDSBA					-1.06 (0.1)	-1.11 (0.2)	-0.41 (01)
ΔPAH, cm	6.4 (2.2)	0.5 (4.4)	1.4 (4.4)	4.4 (3.5)			
Testic vol, ml			14.3 (3.3)	14.0 (2.7)			
Tanner G	5/0/1/1/3	3/3/2/2/0	0/0/4/12/1	0/1/7/10/3	4-5		
At (near-)AH							
Age	23.3 (4.0)	21.7 (3.1)			17.4 (0.2)		
Hgt, cm	164.8 (4.0)	163.7 (3.7)			164.1 (1.6)	164.8 (1.6)	166.9 (1.5)
NAH-TH					-7.8 (1.6)	-5.3 (1.3)	-4.5 (1.4)
ΔHgt, cm					18.2 (1.6)	20.6 (1.5)	22.5 (1.4)
ΔHgt 3yrs					23.8 (2.3)	26.7 (2.0)	30.7 (1.1)
ΔHgt 2yrs					14.7 (1.5)	17.8 (1.6)	19.9 (1.4)
Hgt SDS	-2.6 (0.7)	-2.7 (0.7)			-1.4 (0.1)	-1.4 (0.2)	-1.0 (0.1)
BA, yrs	18.5 (0.7)	18.7 (0.7)			15.3 (0.1)		
Testic vol, ml	12.8 (3.0)	12.2 (3.5)					

**Data from Mauras et al. are expressed as mean (SE). Data from other papers are expressed as mean (SD).*

#Derived from Hero et al, 2005 on 16 and 14 patients, respectively.

AH, adult height; BA, bone age; G, genital stage according to Tanner; GH, growth hormone; Hgt, height; HV, height velocity; HSDSBA, height SDS for bone age; ISS, idiopathic short stature; NAH, near-adult height; PAH, predicted adult height; SDS, standard deviation score; T, testosterone; testic, testicular; TH, target height; vol, volume; yrs, years.

Unfortunately, no AH data could be collected. Furthermore, the interpretation of the results is complicated because of a potential selection bias, since the number of patients who could be analyzed decreased from the original 52 to 41 and 28 completing 2 and 3 years, respectively, without information on the distribution per group.

An interesting approach was taken by a French group (126), which explored the effect on AH of rhGH plus anastrozole, compared with rhGH alone and historical untreated controls, by the end of puberty in boys with ISS (**Table 3**). In this small study, rhGH plus anastrozole, despite being started at such late stage of puberty, seemed to allow boys with ISS to reach a greater AH than rhGH alone (LoE 2).

3.2.1.3 Letrozole or Anastrozole

As mentioned previously, first year data from a direct comparison of anastrozole and letrozole in children with ISS confirmed that letrozole is more potent in hormonal manipulation, but suggested that PAH increased more in the anastrozole group (118) (LoE 3). Unfortunately, long-term results of this study have not been reported, but even if these would be available, the lack of an untreated control group would have hampered the interpretation.

An RCT in 76 boys with ISS consisted of three arms: 1) AI (letrozole or anastrozole); 2) rhGH, and; 3) AI plus rhGH (103). The authors concluded that AI plus rhGH for 24–36 months increased height potential in pubertal boys with ISS more than rhGH or AI alone (LoE 1), but unfortunately no data have been reported on attained AH. The effect was considerably greater if patients were treated for at least 36 months, but this could only be investigated in the 19 out of 54 boys with a residual height potential at 24 months who chose to continue treatment, resulting in potentially selective loss to follow up. As mentioned earlier, the study was not powered to investigate

TABLE 3 | Effect of anastrozole (Ana) or letrozole (Let) in boys with growth hormone deficiency (GHD) or idiopathic short stature (ISS).

Country, Dg	**US, GHD**		**France, ISS**			**US, GHD versus ISS**	
Reference	**(125)**		**(126)**			**(127)**	
Medication	**Ana+GH**	**Placebo+GH**	**Ana+GH**	**GH**	**Controls**	**GH+[Ana/Let]**	**GH+[Ana/Let]**
N	26	26	12	12	17	115	27
At start							
Age, yrs	13.8 (0.3)	14.2 (0.2)	15.2 (0.8)	15.2 (1.1)	15.1 (0.8)	14.7 (1.9)	13.8 (1.7)
Hgt, cm	149.7 (1.6)	151.6 (13)	155.0 (4)	156.3 (2.9)	156.1(3.5)		
Hgt SDS	-1.4 (0.2)	-1.5 (0.2)	-1.7 (0.7)	-1.7 (1)	-1.7 (0.8)	-1.0 (0.9)	-1.0 (0.8)
TH, cm	169.8 (1.6)	173.1 (1.2)	-1.15	-1.2	-1.15		
BA, yrs	13.7 (0.2)	13.4 (0.2)	14.5 (0.8)	14.6 (0.6)	14.6 (0.7)	13.5 (2.4)	13.5 (1.0)
BA/CA						0.97 (0.10)	0.99 (0.10)
PAH, cm			157.9 (3.8)	158.2 (2.9)			
PAH SDS			-2.9 (0.6)	-2.84 (0.5)			
Testic vol, ml			22.2 (5)	22.4 (8)	22 (5)		
Tanner G	2-4	2-4					
T, nmol/L	7.5 (1.2)	5.6 (1.0)	5.6 (0.9)	5.5 (0.9)	5.5 (0.8)		
At 1-2 yrs	**At 2 yrs**			**At ≈1 yr (NAH)**		**At 1 yr**	
N	? (total 41)		12	12		72	19
Duration GH	2 yrs	2 yrs	19 (5.9)mo	11.5 (5)mo			
Age, yrs			16.8 (0.7)	16.2 (1.1)			
Hgt, cm	162.9 (1.4)	166.6 (1.4)	168.4 (2.6)	164.2(5.6)	160.1(2.8)		
Hgt SDS 0						-0.92 (0.9)	-0.87 (0.9)
Hgt SDS			-1.1 (0.9)	-1.8 (0.9)		-0.62 (1.0)	-0.69 (0.8)
Δ hgt,cm			12.7 (5.6)	7.8 (5)			
BA, yrs	15.4 (0.2)	16.0 (0.2)					
BA/CA						0.93 (0.1)	0.96 (0.1)
ΔBA, yrs	1.8 (0.1)	2.7 (0.1)					
ΔHSDSBA	0.5 (0.1)	0.0 (0.1)					
ΔPAH, cm	4.5 (1.2)	1.1 (1.1)					
NAH-PAH, cm			10.5 (5.2)	5.9 (4.5)			
T, nmol/L	21.8 (2.5)	19.8 (2.0)					
At 2-3 yrs	**At 3 yrs**					**At 2 yrs**	
N	? (total 28)					27	9
Hgt, cm	165.8 (1.3)	167.8 (1.8)					
Hgt SDS 0						-1.00 (1.0)	-0.85 (0.9)
Hgt SDS						-0.40 (1.2)	-0.65 (0.5)
BA, yrs	15.9 (0.3)	17.2 (0.3)					
ΔBA, yrs	2.5 (0.2)	4.1 (0.1)					
ΔHSDSBA	0.8 (0.2)	-0.1 (0.2)					
BA/CA						0.95 (0.1)	0.96 (0.06)
ΔPAH, cm	6.7 (1.4)	1.0 (1.1)					
T, nmol/L	17.7 (2.2)	11.7 (1.1)					

BA, bone age; CA, chronological age; G, genital stage according to Tanner; Hgt, height; Hgt SDS 0, height SDS at baseline; HSDSBA, height SDS for bone age; mo, months; NAH, near-adult height; PAH, predicted adult height; SDS, standard deviation score; T, testosterone; testic, testicular; TH, target height; vol, volume; yrs, years.

differences between letrozole and anastrozole in terms of efficacy and safety. The total QoL scores increased significantly at 24 months in the rhGH and AI plus rhGH group, but QoL derived from the children's reports did not increase in the AI group, while it increased in all groups in the parents' reports. Increases in QoL scores were associated with increases in height SDS (136).

A retrospective assessment of the effect of anastrozole or letrozole with or without rhGH treatment for a mean period of 2.1 years was performed in 96 adolescent boys with, as stated in the report, an "idiopathic decrease in PAH when compared with TH" (137). In contrast, the baseline data showed that mean pre-treatment PAH was close to mean TH (range -3.4 to +2.4 cm around TH) and mean height SDS ranged between -1.0 and 0.0. The normal stature of these boys and low number of patients who reached NAH (n=22), as well as the retrospective design, preclude firm conclusions (LoE 4).

In an observational study from the United States (127) on boys with GHD or ISS, with a similar loss to follow up and risk of selection bias as mentioned for the RCTs, the authors concluded that the addition of an AI may augment growth potential as indicated by continued height SDS increase with decreased BA/chronological age (BA/CA) ratio (**Table 3**) (LoE 4).

A recent study from China reported on a comparison of the effect of rhGH alone or combined with letrozole or anastrozole for one year or more (138). After intervention, there were significant differences in ΔBA/ΔCA, ΔHeight SDS for BA and ΔPAH between the rhGH plus AI group and the rhGH group (LoE 3). However, multiple adverse events were reported (see later).

3.2.2 Haploinsufficiency of *SHOX*, *NPR2*, and *ACAN*

There are only a few anecdotal reported data on the combination of rhGH plus AI in children with these genetic variants. One child with a heterozygous pathogenic *NPR2* variant was treated with rhGH in a dosage of 50 μg/kg.d and letrozole (2.5 mg/d) since he was 13 years old (83). His height SDS remained stable and his AH prediction based on his bone age improved from 156 cm to 167.4 cm during therapy (LoE 4).

In one case of the large cohort of patients with *ACAN* haploinsufficiency (79), the effect of letrozole treatment for one year was reported, resulting in arrested bone maturation (LoE 4). In a recent Chinese study (86), the effect of GnRHa or an AI in addition to rhGH was compared with rhGH alone, but no separate analysis of these two interventions were presented, as discussed earlier (LoE 4).

3.2.3 Hypothyroidism

There is one case report on the effect of anastrozole as cotreatment to LT4 treatment in a boy who was diagnosed at 12 years of age with a height of -3.5 SDS (139). After 2 years of LT4, anastrozole was added for 1.5 years because of rapid bone maturation, resulting in a PAH of 11 cm below TH. BA advancement slowed and the patient's NAH was 2.4 cm taller than PAH at start of anastrozole (LoE 4).

3.2.4 Chronic Kidney Disease

In a case report, a one year treatment with anastrozole after renal transplantation appeared to have a positive effect on growth and the authors suggested that this strategy should be considered in children who present with significant short stature close to puberty when time for other therapeutic options is limited (140) (LoE 4).

3.3 The effect of Aromatase Inhibitors in Girls

For the four rare disorders characterized by hyperestrogenism (aromatase excess syndrome, Peutz-Jeghers syndrome, McCune-Albright syndrome, and functional follicular ovarian cysts) AIs in girls have been used with modest results (5). Regarding hyperandrogenism, the combination of letrozole and a GnRHa has been applied to 7 girls with CAH and CPP, with apparently a good effect on AH (98) (LoE 4).

Most clinicians are hesitant to use AIs in girls in order to increase AH, and in the most recent review AIs were considered "contraindicated in girls with short stature due to concerns about precipitating ovarian cysts with risk of torsion" (3), based on the occurrence of ovarian torsion in patients with congenital aromatase deficiency. Still, a prospective phase 2a study was performed in 40 girls consecutively referred for early puberty (onset 7.5–9 years) with a PAH <−2 or >1.5 SD lower than their TH, which compared GnRHa plus anastrozole (n=20) with GnRHa alone (n=20) for 2 years or until the age of 10 years (141). In the group receiving the combination treatment, PAH SDS gain was almost double of that observed in the group on GnRHa alone by 12 and 18 months, and reached the maximum of +1.2 SDS (7.5 cm) vs +0.3 SDS (1.9 cm) after 2 years, respectively (LoE 2).

3.4 Safety of Aromatase Inhibitors in Childhood and Adolescence

The use of AIs in male children and adolescents has been associated with several adverse events, probably associated with a decrease of circulating and intracellular estrogens and an increase of circulating androgens due to increased LH secretion, particularly on letrozole.

Estrogen receptors and aromatase activity are ubiquitously present throughout the body, suggesting that estrogen signaling is crucial for many tissues. The main potential adverse events associated with estrogen deficiency are changes in BMD, bone turnover, lipid metabolism, insulin sensitivity, and cognitive performance (1, 3). In fact, adults with estrogen deficiency due to hypogonadism or aromatase deficiency do present with a low BMD. Although in the RCTs no change of BMD was reported (130), a temporary decrease of BMD was noted by Krebs et al. (135).

In the study on the effect of letrozole in boys with ISS, most of whom were prepubertal boys at start of treatment, vertebral anomalies were noted (142). A similar observation was made in a boy treated for 11 years from 4 years of age onward (109). Serum HDL slightly decreased and insulin sensitivity slightly increased (143). No significant adverse effect on cognitive performance was found (1). Increased erythropoiesis was noted in several studies (2, 144–146). A theoretical risk of AIs is that letrozole-induced gonadotropin secretion and ensuing high concentrations of intratesticular testosterone might affect development of seminiferous epithelium (133). In fact, abnormalities of sperm (impaired motility and subnormal morphology) were observed in the boy treated with letrozole for 11 years (109), but not in an RCT on the effect of anastrozole (147).

Multiple adverse events were noted during follow-up of the 151 patients treated with rhGH plus letrozole or anastrozole, including elevated uric acid, decreased HDL, severe acne, excitement, hyperactivity and irritability, a fracture, mild renal dysfunction, inactivity, drowsiness, memory loss and performance decline, mildly abnormal liver function, and granulocytopenia (138). The percentages of knee pain (11-12%) and impaired fasting glucose (1-2%) were similar to those in patients on rhGH alone. In another report (2), the severity of acne and hematocrit significantly increased in boys who started treatment in Tanner IV-V. In girls no adverse events were observed (141).

Although no head-to-head comparison is possible, my impression is that adverse events of letrozole are more frequent than of anastrozole.

4 DISCUSSION AND CONCLUSION

In general, the scientific evidence for the efficacy of GnRHa or AIs in the treatment of various growth disorders is suboptimal, because of the scarcity of randomized controlled trials up to AH and conflicting reports. **Table 4** shows my subjective impression of the efficacy of GnRHa or AI alone, or various combinations, in particular with rhGH.

TABLE 4 | Apparent efficacy of different treatment regimens manipulating skeletal maturation for increasing adult height.

	GnRHa alone	GnRHa + rhGH	GnRHa + AI	AI alone	AI + rhGH
CPP	++	+[1]	±		
Hypothyroidism[2]	±	±			
Laron syndrome	±				
GHD		++[3]			±
SGA		+[3]			
ISS	±	+ in girls, ± in boys		±	±
SHOX, *NPR2*, or *ACAN* haploinsufficiency		±			±
CAH		+[3]	± [3]		±
CDGP				±	±

[1]*In case of severely decreased growth velocity during GnRHa therapy.*
[2]*In case of severe longstanding hypothyroidism and bone age delay.*
[3]*In case of precocious puberty or when entering puberty at a low height SDS.*
++: Treatment considered effective in significantly increasing adult height, based on several good quality studies.
+: Some treatment effect on adult height with reasonable certainty.
+/-: Uncertain treatment effect due to conflicting or limited evidence.
CAH, congenital adrenal hyperplasia; CDGP, constitutional delay of growth and puberty; CPP, central precocious puberty; GHD, growth hormone deficiency; ISS, idiopathic short stature; SGA, small for gestational age.

The efficacy of a GnRHa alone is proven for children with CPP, but less certain for any other condition. The available clinical evidence appears sufficient to conclude that in rhGH-treated children with GHD or SGA and in rhGH-treated girls with ISS who develop CPP or who are short at pubertal onset, AH can be increased by adding a GnRHa for 2-3 years. Anecdotal reports suggest that a larger effect can be reached with a longer treatment period, for example in girls with ISS and relatively early puberty. However, the potential adverse consequences in terms of bone health and psychosocial development have to be considered.

A combination of rhGH plus GnRHa has also been used for children with several conditions, if PAH SDS was low at the onset of puberty. The effect appears similar among conditions, and close to a gain of 1 SD (6-7 cm). However, the number of RCTs is low, much of the evidence is derived from uncontrolled studies and case reports, for most conditions rhGH treatment is not registered, and GnRHas can only be prescribed off-label except for CPP. Still, I believe that the similarity between the results in children with the various conditions, particularly children with CPP on GnRHa treatment with a low height velocity and PAH and those in children with CAH with a low PAH, render it plausible that growth stimulation by rhGH in combination with low estrogen exposure by a GnRHa provides more time for growth by inhibiting maturation of the epiphyseal growth plates and increases AH. These observations are also in line with the observations on experiments of nature (continued growth and tall AH in disorders where circulating estrogens are low). GnRHa treatment is usually tolerated well, and a modest decrease of BMD appears to be compensated after discontinuation of treatment.

Theoretically, one would expect a similar effect of AIs on growth and skeletal maturation as of GnRHas, but the lack of reported data on AH make it difficult to estimate the extent of the effect. AIs do have a therapeutic value in a few very rare disorders, but their value in growth disorders, either alone or in combination with rhGH, is still uncertain. In patients with GHD or ISS, co-medication with anastrozole (103, 125) may have a similar effect as co-medication with a GnRHa (45), but the lack of AH data precludes a firm conclusion. Long-term follow-up of the boys with ISS treated with letrozole have shown that a statistically significant increase of PAH does not always translate to an increased AH (124). Comparing anastrozole and letrozole co-medication, the results tend to be slightly superior for anastrozole, but this has to be confirmed by an RCT comparing the two compounds directly.

AI treatment leads to increased plasma testosterone, which is considered a psychological advantage for adolescent males but a potential disadvantage for females. Therefore, the use of AIs in adolescence has virtually be confined to boys. The preliminary data on the use of anastrozole in short girls (141) challenge this hypothesis, but this observation needs confirmation.

Regarding safety, GnRHas are considered safe (11), but there is more uncertainty about the safety of AIs, particularly if treatment is given for a long period, and possibly more for letrozole than for anastrozole.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

ACKNOWLEDGMENTS

I am grateful to Dr. Christiaan de Bruin and Dr. Sjoerd Joustra (Willem-Alexander Children's Hospital LUMC, Department of Pediatrics, Division of Pediatric Endocrinology) and Prof. Alexander A.L. Jorge (Unidade de Endocrinologia Genetica, Laboratorio de Endocrinologia Celular e Molecular (LIM/25) do Hospital das Clínicas da Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil) for their constructive comments on previous versions of this manuscript.

REFERENCES

1. Hero M. Aromatase Inhibitors in the Treatment of Short Stature. *Endocr Dev* (2016) 30:130–40. doi: 10.1159/000439338
2. Ferris JA, Geffner ME. Are Aromatase Inhibitors in Boys With Predicted Short Stature and/or Rapidly Advancing Bone Age Effective and Safe? *J Pediatr Endocrinol Metab* (2017) 30(3):311–7. doi: 10.1515/jpem-2016-0219
3. Saroufim R, Eugster EA. Non-GH Agents and Novel Therapeutics in the Management of Short Stature. *Indian J Pediatr* (2021) 88(12):1209–13. doi: 10.1007/s12098-021-03824-3
4. Allen NG, Krishna KB, Lee PA. Use of Gonadotropin-Releasing Hormone Analogs in Children. *Curr Opin Pediatr* (2021) 33(4):442–8. doi: 10.1097/MOP.0000000000001026
5. Wit JM, Hero M, Nunez SB. Aromatase Inhibitors in Pediatrics. *Nat Rev Endocrinol* (2012) 8(3):135–47. doi: 10.1038/nrendo.2011.161
6. Wit JM, Oostdijk W. Novel Approaches to Short Stature Therapy. *Best Pract Res Clin Endocrinol Metab* (2015) 29(3):353–66. doi: 10.1016/j.beem.2015.01.003
7. Burns PB, Rohrich RJ, Chung KC. The Levels of Evidence and Their Role in Evidence-Based Medicine. *Plast Reconstr Surg* (2011) 128(1):305–10. doi: 10.1097/PRS.0b013e318219c171
8. Baron J, Savendahl L, De Luca F, Dauber A, Phillip M, Wit JM, et al. Short and Tall Stature: A New Paradigm Emerges. *Nat Rev Endocrinol* (2015) 11(12):735–46. doi: 10.1038/nrendo.2015.165
9. Weise M, De Levi S, Barnes KM, Gafni RI, Abad V, Baron J. Effects of Estrogen on Growth Plate Senescence and Epiphyseal Fusion. *Proc Natl Acad Sci U.S.A.* (2001) 98(12):6871–6. doi: 10.1073/pnas.121180498
10. Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR, Antoniazzi F, et al. Consensus Statement on the Use of Gonadotropin-Releasing Hormone Analogs in Children. *Pediatrics* (2009) 123(4):e752–62. doi: 10.1542/peds.2008-1783
11. Bangalore Krishna K, Fuqua JS, Rogol AD, Klein KO, Popovic J, Houk CP, et al. Use of Gonadotropin-Releasing Hormone Analogs in Children: Update by an International Consortium. *Horm Res Paediatr* (2019) 91(6):357–72. doi: 10.1159/000501336
12. Den Ouden DT, Kroon M, Hoogland PH, Geelhoed-Duijvestijn PH, Wit JM. A 43-Year-Old Male With Untreated Panhypopituitarism Due to Absence of the Pituitary Stalk: From Dwarf to Giant. *J Clin Endocrinol Metab* (2002) 87(12):5430–4. doi: 10.1210/jc.2002-020672
13. Faustini-Fustini M, Balestrieri A, Rochira V, Carani C. The Apparent Paradox of Tall Stature With Hypopituitarism: New Insights From an Old Story. *J Clin Endocrinol Metab* (2003) 88(8):4002–3; author reply 3. doi: 10.1210/jc.2003-030603
14. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, et al. Estrogen Resistance Caused by a Mutation in the Estrogen-Receptor Gene in a Man. *N Engl J Med* (1994) 331(16):1056–61. doi: 10.1056/NEJM199410203311604
15. Conte FA, Grumbach MM, Ito Y, Fisher CR, Simpson ER. A Syndrome of Female Pseudohermaphrodism, Hypergonadotropic Hypogonadism, and Multicystic Ovaries Associated With Missense Mutations in the Gene Encoding Aromatase (P450arom). *J Clin Endocrinol Metab* (1994) 78(6):1287–92. doi: 10.1210/jcem.78.6.8200927
16. Gault EJ, Perry RJ, Cole TJ, Casey S, Paterson WF, Hindmarsh PC, et al. Effect of Oxandrolone and Timing of Pubertal Induction on Final Height in Turner's Syndrome: Randomised, Double Blind, Placebo Controlled Trial. *BMJ* (2011) 342:d1980. doi: 10.1136/bmj.d1980
17. Ross JL, Cassorla FG, Skerda MC, Valk IM, Loriaux DL, Cutler GB. A Preliminary Study of the Effect of Estrogen Dose on Growth in Turner's Syndrome. *N Engl J Med* (1983) 3:1104–6. doi: 10.1056/NEJM198311033091806
18. Caruso-Nicoletti M, Cassorla F, Skerda M, Ross JL, Loriaux DL, Cutler GB Jr. Short Term, Low Dose Estradiol Accelerates Ulnar Growth in Boys. *J Clin Endocrinol Metab* (1985) 61(5):896–8. doi: 10.1210/jcem-61-5-896
19. Ross JL, Long LM, Skerda M, Cassorla F, Loriaux DL, Cutler GBJr. Growth Response Relationship Between Growth Hormone Dose and Short Term Growth in Patients With Turner's Syndrome. *J Clin Endocrinol Metab* (1986) 63(4):1028–30. doi: 10.1210/jcem-63-4-1028
20. Ross JL, Cassorla F, Carpenter G, Long LM, Royster MS, Loriaux DL, et al. The Effect of Short Term Treatment With Growth Hormone and Ethinyl Estradiol on Lower Leg Growth Rate in Girls With Turner's Syndrome. *J Clin Endocrinol Metab* (1988) 67(3):515–8. doi: 10.1210/jcem-67-3-515
21. Ross JL, Quigley CA, Cao D, Feuillan P, Kowal K, Chipman JJ, et al. Growth Hormone Plus Childhood Low-Dose Estrogen in Turner's Syndrome. *N Engl J Med* (2011) 364(13):1230–42. doi: 10.1056/NEJMoa1005669
22. Venn A, Bruinsma F, Werther G, Pyett P, Baird D, Jones P, et al. Oestrogen Treatment to Reduce the Adult Height of Tall Girls: Long-Term Effects on Fertility. *Lancet* (2004) 364(9444):1513–8. doi: 10.1016/S0140-6736(04)17274-7
23. Hendriks AE, Drop SL, Laven JS, Boot AM. Fertility of Tall Girls Treated With High-Dose Estrogen, a Dose-Response Relationship. *J Clin Endocrinol Metab* (2012) 97(9):3107–14. doi: 10.1210/jc.2012-1078
24. Huttunen H, Varimo T, Huopio H, Voutilainen R, Tenhola S, Miettinen PJ, et al. Serum Testosterone and Oestradiol Predict the Growth Response During Puberty Promoting Treatment. *Clin Endocrinol (Oxf)* (2021). doi: 10.1111/cen.14605
25. Kish MA. Guide to Development of Practice Guidelines. *Clin Infect Dis* (2001) 32(6):851–4. doi: 10.1086/319366
26. The AGREE Collaboration. Development and Validation of an International Appraisal Instrument for Assessing the Quality of Clinical Practice Guidelines: The AGREE Project. *Qual Saf Health Care* (2003) 12(1):18–23. doi: 10.1136/qhc.12.1.18
27. Yanovski JA, Rose SR, Municchi G, Pescovitz OH, Hill SC, Cassorla FG, et al. Treatment With a Luteinizing Hormone-Releasing Hormone Agonist in Adolescents With Short Stature. *N Engl J Med* (2003) 348(10):908–17. doi: 10.1056/NEJMoa013555
28. Khawaja N, Owaineh H, Batieha A, Frahid O, El-Khateeb M, Ajlouni KM. The Effect of Gonadotropin-Releasing Hormone Analogue on Final Adult Height in Children With Idiopathic Short Stature. *Med Princ Pract* (2019) 28(6):509–16. doi: 10.1159/000499929
29. Carel JC, Hay F, Coutant R, Rodrigue D, Chaussain JL. Gonadotropin-Releasing Hormone Agonist Treatment of Girls With Constitutional Short Stature and Normal Pubertal Development. *J Clin Endocrinol Metab* (1996) 81(9):3318–22. doi: 10.1210/jcem.81.9.8784090
30. Bouvattier C, Coste J, Rodrigue D, Teinturier C, Carel JC, Chaussain JL, et al. Lack of Effect of GnRH Agonists on Final Height in Girls With Advanced Puberty: A Randomized Long-Term Pilot Study. *J Clin Endocrinol Metab* (1999) 84(10):3575–8. doi: 10.1210/jcem.84.10.6032
31. Cassio A, Cacciari E, Balsamo A, Bal M, Tassinari D. Randomised Trial of LHRH Analogue Treatment on Final Height in Girls With Onset of Puberty Aged 7.5-8.5 Years. *Arch Dis Child* (1999) 81(4):329–32. doi: 10.1136/adc.81.4.329
32. Lazar L, Kauli R, Pertzelan A, Phillip M. Gonadotropin-Suppressive Therapy in Girls With Early and Fast Puberty Affects the Pace of Puberty But Not Total Pubertal Growth or Final Height. *J Clin Endocrinol Metab* (2002) 87(5):2090–4. doi: 10.1210/jcem.87.5.8481
33. Lampit M, Golander A, Guttmann H, Hochberg Z. Estrogen Mini-Dose Replacement During GnRH Agonist Therapy in Central Precocious Puberty: A Pilot Study. *J Clin Endocrinol Metab* (2002) 87(2):687–90. doi: 10.1210/jcem.87.2.8242
34. Vottero A, Pedori S, Verna M, Pagano B, Cappa M, Loche S, et al. Final Height in Girls With Central Idiopathic Precocious Puberty Treated With Gonadotropin-Releasing Hormone Analog and Oxandrolone. *J Clin Endocrinol Metab* (2006) 91(4):1284–7. doi: 10.1210/jc.2005-1693
35. Tanaka T, Naiki Y, Horikawa R. Combined Treatment With Gonadotropin-Releasing Hormone Analog and Anabolic Steroid Hormone Increased Pubertal Height Gain and Adult Height in Boys With Early Puberty for Height. *Clin Pediatr Endocrinol* (2012) 21(2):35–43. doi: 10.1297/cpe.21.35
36. Teng L, Bui H, Bachrach L, Lee P, Gagne N, Deal C, et al. Catch-Up Growth in Severe Juvenile Hypothyroidism: Treatment With a GnRH Analog. *J Pediatr Endocrinol Metab* (2004) 17(3):345–54. doi: 10.1515/JPEM.2004.17.3.345
37. El Kholy M, Elsedfy HH. Effect of GnRH Analogue on Height Potential in Patients With Severe Growth Hormone Insensitivity Syndrome Treated With IGF-I. *J Pediatr Endocrinol Metab* (2011) 24(11-12):983–8. doi: 10.1515/JPEM.2011.348

38. Backeljauw PF, Kuntze J, Frane J, Calikoglu AS, Chernausek SD. Adult and Near-Adult Height in Patients With Severe Insulin-Like Growth Factor-I Deficiency After Long-Term Therapy With Recombinant Human Insulin-Like Growth Factor-I. *Horm Res Paediatr* (2013) 80(1):47–56. doi: 10.1159/000351958
39. Cara JF, Kreiter ML, Rosenfield RL. Height Prognosis of Children With True Precocious Puberty and Growth Hormone Deficiency - Effect of Combination Therapy With Gonadotropin Releasing Hormone Agonist and Growth Hormone. *J Pediatr* (1992) 120:709–15. doi: 10.1016/S0022-3476(05)80232-X
40. Thomas BC, Stanhope R, Leiper AD. Gonadotropin Releasing Hormone Analogue and Growth Hormone Therapy in Precocious and Premature Puberty Following Cranial Irradiation for Acute Lymphoblastic Leukaemia. *Horm Res* (1993) 39(1-2):25–9. doi: 10.1159/000182690
41. Adan L, Souberbielle JC, Zucker JM, Pierre-Kahn A, Kalifa C, Brauner R. Adult Height in 24 Patients Treated for Growth Hormone Deficiency and Early Puberty. *J Clin Endocrinol Metab* (1997) 82(1):229–33. doi: 10.1210/jc.82.1.229
42. Kohn B, Julius JR, Blethen SL. Combined Use of Growth Hormone and Gonadotropin-Releasing Hormone Analogues: The National Cooperative Growth Study Experience. *Pediatrics* (1999) 104(4 Pt 2):1014–8.
43. Mul D, Wit JM, Oostdijk W, Van den Broeck J. The Effect of Pubertal Delay by GnRH Agonist in GH-Deficient Children on Final Height. *J Clin Endocrinol Metab* (2001) 86(10):4655–6. doi: 10.1210/jcem.86.10.7910
44. Sas TC, de Ridder MA, Wit JM, Rotteveel J, Oostdijk W, Reeser HM, et al. Adult Height in Children With Growth Hormone Deficiency: A Randomized, Controlled, Growth Hormone Dose-Response Trial. *Horm Res Paediatr* (2010) 74(3):172–81. doi: 10.1159/000281323
45. Mericq MV, Eggers M, Avila A, Cutler GB Jr, Cassorla F. Near Final Height in Pubertal Growth Hormone (GH)-Deficient Patients Treated With GH Alone or in Combination With Luteinizing Hormone-Releasing Hormone Analog: Results of a Prospective, Randomized Trial. *J Clin Endocrinol Metab* (2000) 85(2):569–73. doi: 10.1210/jcem.85.2.6343
46. Walenkamp MJ, Pereira AM, Oostdijk W, Stokvis-Brantsma WH, Pfaeffle RW, Blankenstein O, et al. Height Gain With Combined Growth Hormone and Gonadotropin-Releasing Hormone Analog Therapy in Two Pubertal Siblings With a Growth Hormone-Releasing Hormone Receptor Mutation. *J Clin Endocrinol Metab* (2008) 93(1):204–7. doi: 10.1210/jc.2007-1572
47. Mericq V, Gajardo H, Eggers M, Avila A, Cassorla F. Effects of Treatment With GH Alone or in Combination With LHRH Analog on Bone Mineral Density in Pubertal GH-Deficient Patients. *J Clin Endocrinol Metab* (2002) 87(1):84–9. doi: 10.1210/jcem.87.1.8148
48. Lazar L, Pollak U, Kalter-Leibovici O, Pertzelan A, Phillip M. Pubertal Course of Persistently Short Children Born Small for Gestational Age (SGA) Compared With Idiopathic Short Children Born Appropriate for Gestational Age (AGA). *Eur J Endocrinol* (2003) 149(5):425–32. doi: 10.1530/eje.0.1490425
49. Hernandez MI, Martinez-Aguayo A, Cavada G, Pena V, Trejo L, Avila A, et al. Accelerated Early Pubertal Progression, Ovarian Morphology, and Ovarian Function in Prospectively Followed Low Birth Weight (LBW) Girls. *J Pediatr Endocrinol Metab* (2013) 26(3-4):223–30. doi: 10.1515/jpem-2012-0345
50. Finken MJJ, van der Steen M, Smeets CCJ, Walenkamp MJE, de Bruin C, Hokken-Koelega ACS, et al. Children Born Small for Gestational Age: Differential Diagnosis, Molecular Genetic Evaluation, and Implications. *Endocr Rev* (2018) 39(6):851–94. doi: 10.1210/er.2018-00083
51. Lem AJ, van der Kaay DC, de Ridder MA, Bakker-van Waarde WM, van der Hulst FJ, Mulder JC, et al. Adult Height in Short Children Born SGA Treated With Growth Hormone and Gonadotropin Releasing Hormone Analog: Results of a Randomized, Dose-Response GH Trial. *J Clin Endocrinol Metab* (2012) 97(11):4096–105. doi: 10.1210/jc.2012-1987
52. van der Steen M, Lem AJ, van der Kaay DC, Hokken-Koelega AC. Puberty and Pubertal Growth in GH-Treated SGA Children: Effects of 2 Years of GnRHa Versus No GnRHa. *J Clin Endocrinol Metab* (2016) 101(5):2005–12. doi: 10.1210/jc.2016-1317
53. van der Kaay D, Bakker B, van der Hulst F, Mul D, Mulder J, Schroor E, et al. Randomized GH Trial With Two Different Dosages in Combination With a GnRH Analogue in Short Small for Gestational Age Children: Effects on Metabolic Profile and Serum GH, IGF1, and IGFBP3 Levels. *Eur J Endocrinol* (2010) 162(5):887–95. doi: 10.1530/EJE-09-1113
54. Lem AJ, Jobse I, van der Kaay DC, de Ridder MA, Raat H, Hokken-Koelega AC. Health-Related Quality of Life in Short Children Born Small for Gestational Age: Effects of Growth Hormone Treatment and Postponement of Puberty. *Horm Res Paediatr* (2012) 77(3):170–9. doi: 10.1159/000337218
55. van der Steen M, Lem AJ, van der Kaay DC, Hokken-Koelega AC. Insulin Sensitivity and Beta-Cell Function in SGA Children Treated With GH and GnRHa: Results of a Long-Term Trial. *J Clin Endocrinol Metab* (2016) 101(2):705–13. doi: 10.1210/jc.2015-3435
56. Goedegebuure WJ, van der Steen M, de With JL, Hokken-Koelega A. Cognition, Health-Related Quality of Life, and Psychosocial Functioning After GH/GnRHa Treatment in Young Adults Born SGA. *J Clin Endocrinol Metab* (2018) 103(11):3931–8. doi: 10.1210/jc.2018-01463
57. Goedegebuure WJ, van der Steen M, Kerkhof GF, Hokken-Koelega ACS. Longitudinal Study on Metabolic Health in Adults SGA During 5 Years After GH With or Without 2 Years of GnRHa Treatment. *J Clin Endocrinol Metab* (2020) 105(8):dgaa287. doi: 10.1210/clinem/dgaa287
58. van Gool SA, Kamp GA, Visser-van Balen H, Mul D, Waelkens JJ, Jansen M, et al. Final Height Outcome After Three Years of Growth Hormone and Gonadotropin-Releasing Hormone Agonist Treatment in Short Adolescents With Relatively Early Puberty. *J Clin Endocrinol Metab* (2007) 92(4):1402–8. doi: 10.1210/jc.2006-2272
59. Adler E, Lambert AS, Bouvattier C, Thomas-Teinturier C, Rothenbuhler A, de Boissieu P, et al. Determinants of Final Height in Patients Born Small for Gestational Age Treated With Recombinant Growth Hormone. *Horm Res Paediatr* (2021) 94(1-2):52–62. doi: 10.1159/000516557
60. Binder G, Liebl M, Woelfle J, Eggermann T, Blumenstock G, Schweizer R. Adult Height and Epigenotype in Children With Silver-Russell Syndrome Treated With GH. *Horm Res Paediatr* (2013) 80(3):193–200. doi: 10.1159/000354658
61. Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, et al. Diagnosis and Management of Silver-Russell Syndrome: First International Consensus Statement. *Nat Rev Endocrinol* (2017) 13(2):105–24. doi: 10.1038/nrendo.2016.138
62. Netchine I, van der Steen M, Lopez-Bermejo A, Koledova E, Maghnie M. New Horizons in Short Children Born Small for Gestational Age. *Front Pediatr* (2021) 9:655931. doi: 10.3389/fped.2021.655931
63. Ranke MB, Reiter EO, Price DA. Idiopathic Growth Hormone Deficiency in KIGS: Selected Aspects. In: MB R, DA Price and EO Reiter, editors. *Growth Hormone Therapy in Pediatrics - 20 Years Of KIGS*. Basel: Karger (2007). p. 116–35.
64. Job JC, Toublanc JE, Landier F. Growth of Short Normal Children in Puberty Treated for 3 Years With Growth Hormone Alone or in Association With Gonadotropin- Releasing Hormone Agonist. *Horm Res* (1994) 41(5-6):177–84. doi: 10.1159/000183889
65. Saggese G, Cesaretti G, Barsanti S, Rossi A. Combination Treatment With Growth Hormone and Gonadotropin-Releasing Hormone Analogs in Short Normal Girls. *J Pediatr* (1995) 126(3):468–73. doi: 10.1016/S0022-3476(95)70473-6
66. Tanaka T, Satoh M, Yasunaga T, Horikawa R, Tanae A, Hibi I. GH and GnRH Analog Treatment in Children Who Enter Puberty at Short Stature. *J Pediatr Endocrinol Metab* (1997) 10(6):623–8. doi: 10.1515/JPEM.1997.10.6.623
67. Toumba M, Kokotsis V, Savva SC, Skordis N. Expensive Therapies in Children: Benefit Versus Cost of Combined Treatment of Recombinant Human Growth Hormone and Gonadotropin-Releasing Hormone Analogue in Girls With Poor Height Potential. *J Pediatr Endocrinol Metab* (2014) 27(3-4):311–6. doi: 10.1515/jpem-2013-0210
68. Balducci R, Toscano V, Mangiantini A, Municchi G, Vaccaro F, Picone S, et al. Adult Height in Short Normal Adolescent Girls Treated With Gonadotropin-Releasing Hormone Analog and Growth Hormone. *J Clin Endocrinol Metab* (1995) 80(12):3596–600. doi: 10.1210/jcem.80.12.8530605
69. Li S, Wang X, Zhao Y, Ji W, Mao J, Nie M, et al. Combined Therapy With GnRH Analogue and Growth Hormone Increases Adult Height in Children With Short Stature and Normal Pubertal Onset. *Endocrine* (2020) 69(3):615–24. doi: 10.1007/s12020-020-02375-5

70. Lanes R, Gunczler P. Final Height After Combined Growth Hormone and Gonadotrophin-Releasing Hormone Analogue Therapy in Short Healthy Children Entering Into Normally Timed Puberty. *Clin Endocrinol (Oxf)* (1998) 49(2):197–202. doi: 10.1046/j.1365-2265.1998.00499.x
71. Pasquino AM, Pucarelli I, Roggini M, Segni M. Adult Height in Short Normal Girls Treated With Gonadotropin-Releasing Hormone Analogs and Growth Hormone. *J Clin Endocrinol Metab* (2000) 85(2):619–22. doi: 10.1210/jcem.85.2.6387
72. Benabbad I, Rosilio M, Tauber M, Paris E, Paulsen A, Berggren L, et al. Growth Hormone in Combination With Leuprorelin in Pubertal Children With Idiopathic Short Stature. *Endocr Connect* (2018) 7(5):708–18. doi: 10.1530/EC-18-0137
73. Lazar L, Levy S, Oron T, Meyerovitch J, de Vries L, Shalitin S, et al. The Beneficial Effect of Combined GH/GnRHa Therapy in Increasing Adult Height Outcome in Children With ISS. *J Clin Endocrinol Metab* (2019) 104 (8):3287–95. doi: 10.1210/jc.2019-00233
74. Visser-van Balen H, Geenen R, Kamp GA, Huisman J, Wit JM, Sinnema G. Long-Term Psychosocial Consequences of Hormone Treatment for Short Stature. *Acta Paediatr* (2007) 96(5):715–9. doi: 10.1111/j.1651-2227.2007.00235.x
75. Tuvemo T, Jonsson B, Gustafsson J, Albertsson-Wikland K, Aronson AS, Hager A, et al. Final Height After Combined Growth Hormone and GnRH Analogue Treatment in Adopted Girls With Early Puberty. *Acta Paediatr* (2004) 93(11):1456–62. doi: 10.1111/j.1651-2227.2004.tb02629.x
76. Mul D, Oostdijk W, Waelkens JJ, Drop SL. Final Height After Treatment of Early Puberty in Short Adopted Girls With Gonadotrophin Releasing Hormone Agonist With or Without Growth Hormone. *Clin Endocrinol (Oxf)* (2005) 63(2):185–90. doi: 10.1111/j.1365-2265.2005.02323.x
77. Scalco RC, Melo SS, Pugliese-Pires PN, Funari MF, Nishi MY, Arnhold IJ, et al. Effectiveness of the Combined Recombinant Human Growth Hormone and Gonadotropin-Releasing Hormone Analog Therapy in Pubertal Patients With Short Stature Due to SHOX Deficiency. *J Clin Endocrinol Metab* (2010) 95(1):328–32. doi: 10.1210/jc.2009-1577
78. Hanley PC, Kanwar HS, Martineau C, Levine MA. Short Stature Is Progressive in Patients With Heterozygous NPR2 Mutations. *J Clin Endocrinol Metab* (2020) 105(10):3190–202. doi: 10.1210/clinem/dgaa491
79. Gkourogianni A, Andrew M, Tyzinski L, Crocker M, Douglas J, Dunbar N, et al. Clinical Characterization of Patients With Autosomal Dominant Short Stature Due to Aggrecan Mutations. *J Clin Endocrinol Metab* (2017) 102 (2):460–9. doi: 10.1210/jc.2016-3313
80. Ranke MB, Wit JM. Growth Hormone - Past, Present and Future. *Nat Rev Endocrinol* (2018) 14(5):285–300. doi: 10.1038/nrendo.2018.22
81. Ogata T, Onigata K, Hotsubo T, Matsuo N, Rappold G. Growth Hormone and Gonadotropin-Releasing Hormone Analog Therapy in Haploinsufficiency of SHOX. *Endocr J* (2001) 48(3):317–22. doi: 10.1507/endocrj.48.317
82. Ke X, Liang H, Miao H, Yang H, Wang L, Gong F, et al. Clinical Characteristics of Short-Stature Patients With an NPR2 Mutation and the Therapeutic Response to rhGH. *J Clin Endocrinol Metab* (2021) 106(2):431–41. doi: 10.1210/clinem/dgaa842
83. Vasques GA, Amano N, Docko AJ, Funari MF, Quedas EP, Nishi MY, et al. Heterozygous Mutations in Natriuretic Peptide Receptor-B (NPR2) Gene as a Cause of Short Stature in Patients Initially Classified as Idiopathic Short Stature. *J Clin Endocrinol Metab* (2013) 98(10):E1636–E44. doi: 10.1210/jc.2013-2142
84. Wang SR, Jacobsen CM, Carmichael H, Edmund AB, Robinson JW, Olney RC, et al. Heterozygous Mutations in Natriuretic Peptide Receptor-B (NPR2) Gene as a Cause of Short Stature. *Hum Mutat* (2015) 36(4):474–81. doi: 10.1002/humu.22773
85. Lin L, Li M, Luo J, Li P, Zhou S, Yang Y, et al. A High Proportion of Novel ACAN Mutations and Their Prevalence in a Large Cohort of Chinese Short Stature Children. *J Clin Endocrinol Metab* (2021) 106(7):e2711–9. doi: 10.1210/clinem/dgab088
86. Liang H, Miao H, Pan H, Yang H, Gong F, Duan L, et al. Growth-Promoting Therapies May Be Useful In Short Stature Patients With Nonspecific Skeletal Abnormalities Caused By Acan Heterozygous Mutations: Six Chinese Cases And Literature Review. *Endocr Pract* (2020) 26(11):1255–68. doi: 10.4158/EP-2019-0518
87. Pasquino AM, Pucarelli I, Segni M, Matrunola M, Cerroni F, Cerrone F. Adult Height in Girls With Central Precocious Puberty Treated With Gonadotropin-Releasing Hormone Analogues and Growth Hormone. *J Clin Endocrinol Metab* (1999) 84(2):449–52. doi: 10.1210/jcem.84.2.5431
88. Pucarelli I, Segni M, Ortore M, Arcadi E, Pasquino AM. Effects of Combined Gonadotropin-Releasing Hormone Agonist and Growth Hormone Therapy on Adult Height in Precocious Puberty: A Further Contribution. *J Pediatr Endocrinol Metab* (2003) 16(7):1005–10. doi: 10.1515/JPEM.2003.16.7.1005
89. Liu S, Liu Q, Cheng X, Luo Y, Wen Y. Effects and Safety of Combination Therapy With Gonadotropin-Releasing Hormone Analogue and Growth Hormone in Girls With Idiopathic Central Precocious Puberty: A Meta-Analysis. *J Endocrinol Invest* (2016) 39(10):1167–78. doi: 10.1007/s40618-016-0486-9
90. Wang M, Zhang Y, Lan D, Hill JW. The Efficacy of GnRHa Alone or in Combination With rhGH for the Treatment of Chinese Children With Central Precocious Puberty. *Sci Rep* (2016) 6:24259. doi: 10.1038/srep24259
91. Fu J, Zhang J, Chen R, Ma X, Wang C, Chen L, et al. Long-Term Outcomes of Treatments for Central Precocious Puberty or Early and Fast Puberty in Chinese Girls. *J Clin Endocrinol Metab* (2020) 105(3):dgz027. doi: 10.1210/jcem.86.4.7412
92. Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, et al. Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* (2010) 95(9):4133–60. doi: 10.1210/jc.2009-2631
93. Bretones P, Riche B, Pichot E, David M, Roy P, Tardy V, et al. Growth Curves for Congenital Adrenal Hyperplasia From a National Retrospective Cohort. *J Pediatr Endocrinol Metab* (2016) 29(12):1379–88. doi: 10.1515/jpem-2016-0156
94. Quintos JB, Vogiatzi MG, Harbison MD, New MI. Growth Hormone Therapy Alone or in Combination With Gonadotropin-Releasing Hormone Analog Therapy to Improve the Height Deficit in Children With Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab* (2001) 86 (4):1511–7. doi: 10.1136/qhc.12.1.18
95. Lin-Su K, Vogiatzi MG, Marshall I, Harbison MD, Macapagal MC, Betensky B, et al. Treatment With Growth Hormone and Luteinizing Hormone Releasing Hormone Analog Improves Final Adult Height in Children With Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab* (2005) 90 (6):3318–25. doi: 10.1210/jc.2004-2128
96. Lin-Su K, Harbison MD, Lekarev O, Vogiatzi MG, New MI. Final Adult Height in Children With Congenital Adrenal Hyperplasia Treated With Growth Hormone. *J Clin Endocrinol Metab* (2011) 96(6):1710–7. doi: 10.1210/jc.2010-2699
97. Longui CA, Kochi C, Calliari LE, Modkovski MB, Soares M, Alves EF, et al. Near-Final Height in Patients With Congenital Adrenal Hyperplasia Treated With Combined Therapy Using GH and GnRHa. *Arq Bras Endocrinol Metabol* (2011) 55(8):661–4. doi: 10.1590/S0004-27302011000800023
98. Juan L, Huamei M, Zhe S, Yanhong L, Hongshan C, Qiuli C, et al. Near-Final Height in 82 Chinese Patients With Congenital Adrenal Hyperplasia Due to Classic 21-Hydroxylase Deficiency: A Single-Center Study From China. *J Pediatr Endocrinol Metab* (2016) 29(7):841–8. doi: 10.1515/jpem-2015-0406
99. Xi W, Mao J, Li S, Zhao Y, Nie M, Yu B, et al. Aromatase Inhibitor Increases the Height of Patients With Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency. *Endocr Pract* (2020) 26(9):997–1002. doi: 10.4158/EP-2019-0610
100. Quintos JB, Salas M. Use of Growth Hormone and Gonadotropin Releasing Hormone Agonist in Addition to L-Thyroxine to Attain Normal Adult Height in Two Patients With Severe Hashimoto's Thyroiditis. *J Pediatr Endocrinol Metab* (2005) 18(5):515–21. doi: 10.1515/JPEM.2005.18.5.515
101. Sendur MA, Aksoy S, Zengin N, Altundag K. Comparative Efficacy Study of 5-Year Letrozole or Anastrozole in Postmenopausal Hormone Receptor-Positive Early Breast Cancer. *J BUON* (2013) 18(4):838–44.
102. Ellis MJ, Suman VJ, Hoog J, Lin L, Snider J, Prat A, et al. Randomized Phase II Neoadjuvant Comparison Between Letrozole, Anastrozole, and Exemestane for Postmenopausal Women With Estrogen Receptor-Rich Stage 2 to 3 Breast Cancer: Clinical and Biomarker Outcomes and Predictive Value of the Baseline PAM50-Based Intrinsic Subtype–ACOSOG Z1031. *J Clin Oncol* (2011) 29(17):2342–9. doi: 10.1200/JCO.2010.31.6950

103. Mauras N, Ross JL, Gagliardi P, Yu YM, Hossain J, Permuy J, et al. Randomized Trial of Aromatase Inhibitors, Growth Hormone, or Combination in Pubertal Boys With Idiopathic, Short Stature. *J Clin Endocrinol Metab* (2016) 101(12):4984–93. doi: 10.1210/jc.2016-2891
104. Simoes-Pereira J, Santos F, Lopes L, Limbert C. Prepubertal Gynaecomastia in a Boy With Peutz-Jeghers Syndrome: Managing the Aromatase Overexpression. *J Pediatr Endocrinol Metab* (2018) 31(10):1149–54. doi: 10.1515/jpem-2017-0455
105. Leschek EW, Flor AC, Bryant JC, Jones JV, Barnes KM, Cutler GBJr. Effect of Antiandrogen, Aromatase Inhibitor, and Gonadotropin-Releasing Hormone Analog on Adult Height in Familial Male Precocious Puberty. *J Pediatr* (2017) 190:229–35. doi: 10.1016/j.jpeds.2017.07.047
106. Lane LC, Flowers J, Johnstone H, Cheetham T. Adult Height in Patients With Familial Male-Limited Precocious Puberty and the Role of an Aromatase Inhibitor in Patient Management. *J Pediatr Endocrinol Metab* (2018) 31(5):551–60. doi: 10.1515/jpem-2017-0363
107. Merke DP, Keil MF, Jones JV, Fields J, Hill S, Cutler GBJr. Flutamide, Testolactone, and Reduced Hydrocortisone Dose Maintain Normal Growth Velocity and Bone Maturation Despite Elevated Androgen Levels in Children With Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab* (2000) 85(3):1114–20. doi: 10.1210/jcem.85.3.6462
108. Goedegebuure WJ, Hokken-Koelega ACS. Aromatase Inhibitor as Treatment for Severely Advanced Bone Age in Congenital Adrenal Hyperplasia: A Case Report. *Horm Res Paediatr* (2019) 92(3):209–13. doi: 10.1159/000501746
109. Atay Z, Turan S, Bugdayci O, Guran T, Bereket A. Restoration of Height After 11 Years of Letrozole Treatment in 11beta-Hydroxylase Deficiency. *Horm Res Paediatr* (2019) 92(3):203–8. doi: 10.1159/000501456
110. Nour MA, Pacaud D. Height Augmentation in 11beta-Hydroxylase Deficiency Congenital Adrenal Hyperplasia. *Int J Pediatr Endocrinol* (2015) 2015(1):12. doi: 10.1186/s13633-015-0008-0
111. Hawton K, Walton-Betancourth S, Rumsby G, Raine J, Dattani M. Growth Hormone With Aromatase Inhibitor May Improve Height in CYP11B1 Congenital Adrenal Hyperplasia. *Pediatrics* (2017) 139(2):e20160730. doi: 10.1542/peds.2016-0730
112. Kanakis GA, Nordkap L, Bang AK, Calogero AE, Bartfai G, Corona G, et al. EAA Clinical Practice Guidelines-Gynecomastia Evaluation and Management. *Andrology* (2019) 7(6):778–93. doi: 10.1111/andr.12636
113. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase Deficiency in Male and Female Siblings Caused by a Novel Mutation and the Physiological Role of Estrogens. *J Clin Endocrinol Metab* (1995) 80 (12):3689–98. doi: 10.1210/jcem.80.12.8530621
114. Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, et al. Effect of Testosterone and Estradiol in a Man With Aromatase Deficiency. *N Engl J Med* (1997) 337(2):91–5. doi: 10.1056/NEJM199707103370204
115. Bilezikian JP, Morishima A, Bell J, Grumbach MM. Increased Bone Mass as a Result of Estrogen Therapy in a Man With Aromatase Deficiency. *N Engl J Med* (1998) 339(9):599–603. doi: 10.1056/NEJM199808273390905
116. Herrmann BL, Saller B, Janssen OE, Gocke P, Bockisch A, Sperling H, et al. Impact of Estrogen Replacement Therapy in a Male With Congenital Aromatase Deficiency Caused by a Novel Mutation in the CYP19 Gene. *J Clin Endocrinol Metab* (2002) 87(12):5476–84. doi: 10.1210/jc.2002-020498
117. McGrath N, O'Grady MJ. Aromatase Inhibitors for Short Stature in Male Children and Adolescents. *Cochrane Database Syst Rev* (2015) 10): CD010888. doi: 10.1002/14651858.CD010888.pub2
118. Neely EK, Kumar RB, Payne SL, Ranadive SA, Suchet DI. Letrozole vs Anastrozole for Height Augmentation in Short Pubertal Males: First Year Data. *J Clin Endocrinol Metab* (2014) 99(11):4086–93. doi: 10.1210/jc.2014-2432
119. Shulman DI, Francis GL, Palmert MR, Eugster EA. Use of Aromatase Inhibitors in Children and Adolescents With Disorders of Growth and Adolescent Development. *Pediatrics* (2008) 121(4):e975–e83. doi: 10.1542/peds.2007-2081
120. Geffner ME. For Debate: Aromatase Inhibitors to Augment Height: Have We Lost Our Inhibitions? *Pediatr Endocrinol Rev* (2008) 5(3):756–9.
121. Geffner ME. Aromatase Inhibitors to Augment Height: Continued Caution and Study Required. *J Clin Res Pediatr Endocrinol* (2009) 1(6):256–61. doi: 10.4274/jcrpe.v1i6.256
122. Dunkel L. Off-Label Use of Aromatase Inhibitors to Promote Taller Stature: Is it Safe. *Horm Res Paediatr* (2010) 74(6):436–7. doi: 10.1159/000317434
123. Hero M, Norjavaara E, Dunkel L. Inhibition of Estrogen Biosynthesis With a Potent Aromatase Inhibitor Increases Predicted Adult Height in Boys With Idiopathic Short Stature: A Randomized Controlled Trial. *J Clin Endocrinol Metab* (2005) 90(12):6396–402. doi: 10.1210/jc.2005-1392
124. Varimo T, Toiviainen-Salo S, Raivio T, Kerttula L, Dunkel L, Hero M. Letrozole Monotherapy in Pre- and Early-Pubertal Boys Does Not Increase Adult Height. *Front Endocrinol (Lausanne)* (2019) 10:201. doi: 10.3389/fendo.2019.00201
125. Mauras N, Gonzalez de PL, Hsiang HY, Desrosiers P, Rapaport R, Schwartz ID, et al. Anastrozole Increases Predicted Adult Height of Short Adolescent Males Treated With Growth Hormone: A Randomized, Placebo-Controlled, Multicenter Trial for One to Three Years. *J Clin Endocrinol Metab* (2008) 93 (3):823–31. doi: 10.1210/jc.2007-1559
126. Rothenbuhler A, Linglart A, Bougneres P. A Randomized Pilot Trial of Growth Hormone With Anastrozole Versus Growth Hormone Alone, Starting at the Very End of Puberty in Adolescents With Idiopathic Short Stature. *Int J Pediatr Endocrinol* (2015) 2015(1):4. doi: 10.1186/1687-9856-2015-4
127. Miller BS, Ross J, Ostrow V. Height Outcomes in Children With Growth Hormone Deficiency and Idiopathic Short Stature Treated Concomitantly With Growth Hormone and Aromatase Inhibitor Therapy: Data From the ANSWER Program. *Int J Pediatr Endocrinol* (2020) 2020:19. doi: 10.1186/s13633-020-00089-z
128. Wickman S, Sipila I, Ankarberg-Lindgren C, Norjavaara E, Dunkel L. A Specific Aromatase Inhibitor and Potential Increase in Adult Height in Boys With Delayed Puberty: A Randomised Controlled Trial. *Lancet* (2001) 357 (9270):1743–8. doi: 10.1016/S0140-6736(00)04895-9
129. Hero M, Wickman S, Dunkel L. Treatment With the Aromatase Inhibitor Letrozole During Adolescence Increases Near-Final Height in Boys With Constitutional Delay of Puberty. *Clin Endocrinol (Oxf)* (2006) 64(5):510–3. doi: 10.1111/j.1365-2265.2006.02499.x
130. Hero M, Makitie O, Kroger H, Nousiainen E, Toiviainen-Salo S, Dunkel L. Impact of Aromatase Inhibitor Therapy on Bone Turnover, Cortical Bone Growth and Vertebral Morphology in Pre- and Peripubertal Boys With Idiopathic Short Stature. *Horm Res* (2009) 71(5):290–7. doi: 10.1159/000208803
131. Salehpour S, Alipour P, Razzaghy-Azar M, Ardeshirpour L, Shamshiri A, Monfared MF, et al. A Double-Blind, Placebo-Controlled Comparison of Letrozole to Oxandrolone Effects Upon Growth and Puberty of Children With Constitutional Delay of Puberty and Idiopathic Short Stature. *Horm Res Paediatr* (2010) 74(6):428–35. doi: 10.1159/000315482
132. Rohani F, Asadi R, Mirboluk AA, Soheilipour F. Letrozole Effect on Final Height of Patients With Constitutional Delay of Growth and Puberty. *Med Arch* (2019) 73(5):307–10. doi: 10.5455/medarh.2019.73.307-310
133. Varimo T, Huopio H, Kariola L, Tenhola S, Voutilainen R, Toppari J, et al. Letrozole Versus Testosterone for Promotion of Endogenous Puberty in Boys With Constitutional Delay of Growth and Puberty: A Randomised Controlled Phase 3 Trial. *Lancet Child Adolesc Health* (2019) 3(2):109–20. doi: 10.1016/S2352-4642(18)30377-8
134. Zhou P, Shah B, Prasad K, David R. Letrozole Significantly Improves Growth Potential in a Pubertal Boy With Growth Hormone Deficiency. *Pediatrics* (2005) 115(2):e245–e8. doi: 10.1542/peds.2004-1536
135. Krebs A, Moske-Eick O, Doerfer J, Roemer-Pergher C, Werf-Grohmann N, Schwab KO. Marked Increase of Final Height by Long-Term Aromatase Inhibition in a Boy With Idiopathic Short Stature. *J Pediatr Endocrinol Metab* (2012) 25(5-6):581–5. doi: 10.1515/jpem-2011-0435
136. Bullinger M, Bloemeke J, Mericq V, Sommer R, Gaete X, Ross JL, et al. Quality of Life in Adolescent Boys With Idiopathic Short Stature: Positive Impact of Growth Hormone and Aromatase Inhibitors. *Horm Res Paediatr* (2018) 90(6):381–92. doi: 10.1159/000496353
137. Pedrosa LF, de Oliveira JM, Thome PRV, Kochi C, Damiani D, Longui CA. Height Increment and Laboratory Profile of Boys Treated With Aromatase Inhibitors With or Without Growth Hormone. *Horm Metab Res* (2017) 49 (10):778–85. doi: 10.1055/s-0043-116944
138. Kong Y, Chen H, Liang L, Zheng M, Fang Y, Wang C. Aromatase Inhibitors Combined With Growth Hormone in Treatment of Adolescent Boys With Short Stature. *Zhejiang Da Xue Xue Bao Yi Xue Ban* (2020) 49(3):283–90. doi: 10.3785/j.issn.1008-9292.2020.04.12
139. Hodax JK, Topor LS, Bialo SR, Quintos JB. Anastrozole Improves Final Adult Height in Severe Hypothyroidism With Rapid Pubertal Progression. *J Endocr Soc* (2021) 5(5):bvab025. doi: 10.1210/jendso/bvab025

140. Mendley SR, Spyropoulos F, Counts DR. Short Stature in Chronic Kidney Disease Treated With Growth Hormone and an Aromatase Inhibitor. *Case Rep Pediatr* (2015) 2015:738571. doi: 10.1155/2015/738571
141. Papadimitriou DT, Dermitzaki E, Papagianni M, Papaioannou G, Papaevangelou V, Papadimitriou A. Anastrozole Plus Leuprorelin in Early Maturing Girls With Compromised Growth: The "GAIL" Study. *J Endocrinol Invest* (2016) 39(4):439–46. doi: 10.1007/s40618-015-0399-z
142. Hero M, Toiviainen-Salo S, Wickman S, Makitie O, Dunkel L. Vertebral Morphology in Aromatase Inhibitor-Treated Males With Idiopathic Short Stature or Constitutional Delay of Puberty. *J Bone Miner Res* (2010) 25(7):1536–43. doi: 10.1002/jbmr.56
143. Hero M, Ankarberg-Lindgren C, Taskinen MR, Dunkel L. Blockade of Oestrogen Biosynthesis in Peripubertal Boys: Effects on Lipid Metabolism, Insulin Sensitivity, and Body Composition. *Eur J Endocrinol* (2006) 155 (3):453–60. doi: 10.1530/eje.1.02226
144. Hero M, Wickman S, Hanhijarvi R, Siimes MA, Dunkel L. Pubertal Upregulation of Erythropoiesis in Boys Is Determined Primarily by Androgen. *J Pediatr* (2005) 146(2):245–52. doi: 10.1016/j.jpeds.2004.09.002
145. Diaz-Thomas A, Shulman D. Use of Aromatase Inhibitors in Children and Adolescents: What's New? *Curr Opin Pediatr* (2010) 22(4):501–7. doi: 10.1097/MOP.0b013e32833ab888
146. Shams K, Cameo T, Fennoy I, Hassoun AA, Lerner SE, Aranoff GS, et al. Outcome Analysis of Aromatase Inhibitor Therapy to Increase Adult Height in Males With Predicted Short Adult Stature and/or Rapid Pubertal Progress: A Retrospective Chart Review. *J Pediatr Endocrinol Metab* (2014) 27(7-8):725–30. doi: 10.1515/jpem-2013-0470
147. Mauras N, Bell J, Snow BG, Winslow KL. Sperm Analysis in Growth Hormone-Deficient Adolescents Previously Treated With an Aromatase Inhibitor: Comparison With Normal Controls. *Fertil Steril* (2005) 84 (1):239–42. doi: 10.1016/j.fertnstert.2005.02.012

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Endocrinology

REVIEW
published: 24 December 2021
doi: 10.3389/fendo.2021.811846

Safety of Pediatric rhGH Therapy: An Overview and the Need for Long-Term Surveillance

Stefano Cianfarani [1,2,3]*

[1] Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy, [2] Dipartimento Pediatrico Universitario Ospedaliero, IRCCS "Bambino Gesù" Children's Hospital, Rome, Italy, [3] Department of Women's and Children's Health, Karolinska Institute and University Hospital, Stockholm, Sweden

Growth hormone (GH) therapy dates back to 1958 and, though has shown an excellent safety profile in the short-term, has never ceased to raise concern about potential long-term side effects. In the last decade, a number of observational studies in different cohorts of young adult patients treated with GH during childhood have yielded conflicting results. The attention has mainly focused on three major potential risks associated with GH therapy: cancer, cardio and cerebrovascular diseases and diabetes. This review intends to provide a detailed overview of the main studies reporting long-term safety in subjects treated with rhGH therapy during childhood, highlighting the evidence for or against the risk of cancer, cardio and cerebrovascular diseases and diabetes.

Keywords: growth hormome, GH deficiency (GHD), IGF - I, gh therapy, hypopituitarism

OPEN ACCESS

Edited by:
Martin Savage,
Queen Mary University of London,
United Kingdom

Reviewed by:
Alan David Rogol,
University of Virginia, United States
George Arthur Werther,
Royal Children's Hospital, Australia

***Correspondence:**
Stefano Cianfarani
stefano.cianfarani@uniroma2.it

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *09 November 2021*
Accepted: *06 December 2021*
Published: *24 December 2021*

Citation:
Cianfarani S (2021) Safety of Pediatric rhGH Therapy: An Overview and the Need for Long-Term Surveillance. Front. Endocrinol. 12:811846. doi: 10.3389/fendo.2021.811846

INTRODUCTION

Growth Hormone (GH) was initially purified from ox pituitaries (1) and thereafter successfully introduced in the treatment of children with GH deficiency in the middle of the last century (2) Human GH (hGH) was extracted from human pituitaries (pit-hGH) making it extremely difficult to find and stimulating the establishment of national agencies in many countries with the purpose to collect human pituitaries for extracting, purifying and distributing pit-hGH for the treatment of children with GH deficiency. Due to the shortage of raw material (i.e. human pituitaries), the therapeutic regimen of pit-hGH was far from optimal, being based on two to three intramuscular injections per week. Nevertheless, pit-hGH replacement therapy was extremely effective in inducing a robust and prolonged catch-up growth in children with hypopituitarism (3, 4).

pit-hGH therapy was continued until 1985 when the first three cases of Creutzfeldt—Jakob disease in young patients who had received pit-hGH injections during childhood were reported (5, 6). The cause of Creutzfeldt—Jakob disease is an infectious agent, termed prion, a misfolded protein able to induce neurodegeneration. As Creutzfeldt—Jakob disease usually affects older adults, these cases raised the suspicion of contamination of pit-hGH. This suspicion was later confirmed and between 1985 and 2003, over 200 cases were reported, mainly in France, United Kingdom, and United States (7).

Meanwhile, recombinant technology developed (8) and, in 1985, the first recombinant human GH (rhGH) produced from E. coli was approved by the FDA in the USA for the treatment of GH

deficiency in childhood. Due to the safety concerns, pit-hGH was banned from the market and replaced by rhGH.

The virtually unlimited supply of rhGH led to the expansion of indications for rhGH therapy, now including childhood and adult GH deficiency, Turner syndrome, chronic renal failure, small for gestational age (SGA), Prader–Willi syndrome, Noonan syndrome, SHOX deficiency, idiopathic short stature (ISS), achondroplasia, short bowel syndrome and HIV wasting syndrome (9). This expansion of rhGH indications has not been associated with increased incidence of serious side effects. However, it has to be pointed out that the vast majority of available observational studies reporting on rhGH safety are short-term and not independent of Pharmaceutical Companies.

The aim of this review is to provide an overview of the main studies reporting long-term safety in subjects treated with rhGH therapy during childhood, focusing on the three major long-term concerns regarding rhGH therapy, namely cancer, cardio and cerebrovascular diseases and diabetes.

CANCER RISK IN PATIENTS TREATED WITH rhGH DURING CHILDHOOD

The experimental evidence supporting a role of GH and insulin-like growth factors (IGF-I and -II) in the development, expansion and dissemination of tumors is robust and based on countless data obtained in cell lines and animals, reported in detail in previous reviews (10–13). The epidemiological evidence linking circulating levels of IGF-I with increased risk of certain tumors, though less robust than experimental data, supports the role of exposure to high levels of IGFs in cancer risk (10). Consistently, the observation that congenital IGF-I deficiency confers protection from cancer, clearly indicates that low IGF-I levels are associated with reduced cancer risk (14–16).

Concern about the risk of cancer in children treated with GH was raised for the first time by case reports describing children who developed leukemia during or following GH treatment in Japan (17–19). Later analysis showed that at least half of these patients had conditions predisposing to leukemia, thus leading to overestimation of the frequency of malignancy (20). On the other hand, the national Cooperative Growth study, a nationwide study in USA initiated in 1985, did not show an increased risk of leukemia in children treated with GH (21).

Nevertheless, concern over a potential increase in cancer risk associated with GH therapy stimulated further observational studies. In 2002, a long-term study reporting data from 1,848 patients treated with pit-hGH during childhood and early adulthood, showed an increased risk of colorectal cancer and Hodgkin lymphoma (HL) (22). However, the absolute number of recorded deaths and cases was extremely low, though statistically significant (2 deaths for colorectal cancer with an expected number of 0.19 and two deaths for HL with an expected number of 0.18). Moreover, almost half of the study cohort had conditions different from idiopathic GH deficiency, including neoplasms and diseases predisposing to cancer.

In 2009, an EU funded (FP7-HEALTH) consortium of eight European countries (Safety and Appropriateness of GH treatments in Europe, SAGHE) was established with the purpose of evaluating long-term safety of rhGH therapy in childhood (23).

In 2012, preliminary and opposite results from different SAGHE cohorts were published (24, 25). In the French cohort comprising 6,500 young adult subjects treated with rhGH during childhood for the indications of isolated GH deficiency (IGHD), short stature associated with small for gestational age (SGA), or idiopathic short stature (ISS) a significant increase in mortality for bone was observed (24). On the contrary, in the same diagnostic cohorts (overall 2,500 patients) from Belgium, Sweden and The Netherlands, not a single case of death from cancer was observed (25).

In 2014, we carried out a systematic review and meta-analysis of studies reporting long-term safety data of rhGH therapy during childhood (26). The standard mortality ratio (SMR) for cancer was not significantly increased whereas overall cancer standard incidence ratio (SIR, 2.74; 95% confidence interval [CI], 1.18–4.41) was higher than reference populations. However, the analysis was based only on few available studies that, in addition, were affected by a number of confounders and biases.

In the GeNeSIS (Genetics and Neuroendocrinology of Short Stature International Study) observational study sponsored by Eli Lilly and conducted on more than 20,000 rhGH-treated patients with different diagnoses, no significant increase in cancer mortality was observed in IGHD, ISS, and SGA patients (27). It has to be pointed out that mean duration of follow-up in this study was 4.2 years only.

The study reporting mortality and morbidity for cancer from the entire dataset of all eight countries of the SAGHE consortium was published in 2017 (28). The patients were classified into three different classes of risk: (1) low risk: isolated growth failure, including IGHD, ISS and SGA; (2) high risk: including patients with previous history of cancer; (3) intermediate risk: non-isolated growth failure and non-cancer patients, including all the other patients with different diagnoses (Turner syndrome, Noonan syndrome etc.). 23,984 patients were enrolled for cancer mortality risk and 10,406 for cancer incidence. The average follow-up time for mortality was 16.5 years per patient, and for cancer incidence 14.8 years per patient. Both mortality and morbidity for cancer were not increased in the low risk cohort whereas SMR and SIR for almost all types of cancers were significantly increased in the high-risk group. The incidence of bone and bladder cancers was significantly raised in the intermediate risk cohort. No relationship between cancer risk and duration or cumulative dose of rhGH was found. In the high-risk cohort, cancer mortality risk increased significantly with increasing daily rhGH dose. Finally, the incidence of HL increased with time (28).

The French SAGHE cohort was then examined in a separate publication (29). Patients were followed for an average of 17.4 ± 5.3 years to a mean age of 28.4 ± 6.2 years.The overall incidence and mortality of cancer were not increased with the exception of

bone tumors (SIR 3.5, 95% CI 1.1- 8.1; SMR 5.0, 95% CI 1.0- 14.6).

The study reporting data from the entire SAGHE cohort with more than 400 000 patient-years and up to 25 years of follow-up, focused on long-term overall and cause-specific mortality in young adult patients treated with recombinant human growth hormone during childhood (30). This study showed no increase in mortality for neoplasms in the low risk group (IGHD, ISS and SGA patients).

FINAL REMARKS ON CANCER RISK IN PATIENTS TREATED WITH rhGH DURING CHILDHOOD

The majority of observational studies, with the exception of the French SAGHE cohort, do not indicate that rhGH therapy affects the risk of cancer in children without other risk factors at least in the 15-16 years following rhGH therapy (**Table 1**) (31). All the available reports are affected by a series of confounders and biases. The study cohorts are often heterogenous, relatively small and observed over a short follow-up. The quality of study designs is different, some reporting data from death certificates and other from detailed analysis of clinical records. Moreover, the absolute rate of events is low, untreated control cohorts are not available, data regarding familial predisposition to cancer and exposure to environmental hazards are lacking as well as local cancer mortality and morbidity indices and information on rhGH dose and treatment duration. Therefore, it is still impossible to draw definitive conclusions on the basis of the available evidence.

There is evidence that GH and IGF-I are not able to directly induce cell transformation and carcinogenesis but may amplify the DNA damaging effects induced by other factors (32, 33). On the other hand, experimental evidence suggests that both GH and IGF-I play a pivotal role in the expansion and dissemination of many tumors thus suggesting a possible accelerator effect in patients who have early stage neoplasms. This potentiality raises concern about the safety of rhGH treatment in patients with previous history of neoplasia, conditions predisposing to cancer (for instance RASopathies including Noonan syndrome) and chromosomal breakage syndromes or DNA-repair disorders, including Fanconi anemia, Bloom syndrome and Down syndrome.

In conclusion, long-term cancer surveillance is still needed in all patients treated with rhGH, especially in those with conditions predisposing to cancer risk and, more in general, in patients who received pharmacological rather than replacement rhGH therapy.

CARDIO AND CEREBROVASCULAR RISK IN PATIENTS TREATED WITH rhGH DURING CHILDHOOD

Acromegaly, a disease characterized by excessive GH secretion, is associated with cardiovascular and cerebrovascular diseases (34). Though acromegalic patients have multiple risk factors that contribute to morbidity and mortality for cardiovascular diseases (CVD) such as hypertension, diabetes and dyslipidemia, nevertheless GH/IGF-1 excess per se may play a role in the increased CVD risk of these patients. Indeed, GH/IGF-1 excess may directly affect endothelial function *via* different mechanisms including: a) endothelial proliferation; b) dysfunction of endothelial progenitor cells; c) induction of oxidative stress; and d) reduction of oxidative defenses (35, 36). Two prospective Dutch and UK-based cohort studies in the elderly and adults respectively, have shown a U-shaped relationship between IGF-I levels and mortality, high circulating IGF-I levels being associated with increased risk of all-cause and CVD mortality (37, 38).

In a Dutch cohort of adult GHD patients on treatment with rhGH, CVD mortality was not increased (39). Data from KIMS, a global, multicenter, non-interventional, pharmaco-epidemiological study in which data were collected from GHD adults receiving rhGH replacement therapy, showed that mortality was slightly but significantly increased especially in women (40). Interestingly, standard mortality ratio (SMR) was significantly associated with IGF-I SDS and among the causes of death, mortality for cerebrovascular disease was significantly increased.

The first study showing increased cardio and cerebrovascular mortality in young adults treated with rhGH during childhood reported data of the French cohort of the SAGHE study (24). The cohort consisted of 6928 young adults with IGHD, neurosecretory dysfunction, ISS and born SGA. SMR was significantly increased for diseases of the circulatory system (SMR 3.07, 95% CI 1.40–5.83) or subarachnoid or intracerebral hemorrhage (SMR 6.66, 95% CI 1.79 –17.05). In contrast to these results, an observational study reporting mortality data from 2543 young adults recruited in the SAGHE cohorts from

TABLE 1 | Summary of the available evidence of cancer, cardio-cerebrovascular and diabetes risk in young adulthood associated with rhGH therapy in childhood.

Cancer risk	Cardio-cerebrovascular risk	Type 2 Diabetes risk
I. **No evidence of increased risk in low risk group (IGHD, ISS and SGA).** II. **Increased risk of bone tumors in the French SAGHE cohort.**	**Evidence of potential increased risk, presumably in association with other risk factors such as family history, environment, lifestyle, ethnicity, comorbidities and, possibly, female gender.**	**Evidence of potential increased risk in presence of other risk factors such as obesity, family history, sedentary lifestyle, comorbidities.**

IGHD, isolated GH deficiency; ISS, idiopathic short stature; SGA, small for gestational age.

Belgium, The Netherlands and Sweden, with the same diagnostic categories of the French cohort, showed not a single case of death for cardio or cerebrovascular diseases (25).

A further report from the French SAGHE study group showed increased cerebrovascular morbidity for hemorrhagic stroke and particularly subarachnoid hemorrhage in 6874 young adults treated with rhGH during childhood for IGHD, ISS and SGA (41).

The study reporting mortality data from the complete dataset of all eight countries of the SAGHE consortium including more than 24000 patients with up to 25 years of follow-up was published in 2020 (30). The results showed that all-cause mortality was not increased in low-risk patients (IGHD and ISS) whereas it was significantly increased in children born small for gestational age, though this result was skewed by the French sub-cohort. Overall mortality was not associated with mean daily or cumulative doses of rhGH for any of the risk groups. Notably, when looking at cause specific mortality, mortality for diseases of circulatory system was significantly increased in all risk groups. A recent large nationwide cohort study conducted in Sweden, included patients treated with rhGH for the indications of IGHD, ISS and SGA (42). The Authors collected data on cardiovascular risk as well as a number of covariates such as gestational age, birth weight, birth length, socioeconomic status, and height. 53,444 individuals (3408 patients and 50036 controls) were followed up for a median of 14.9 years. The adjusted hazard ratio (HR) for all cardiovascular events was significantly increased in patients (HR, 1.69; 95% CI, 1.30-2.19), and particularly in women (HR, 2.05; 95% CI, 1.31-3.20). Each diagnostic category (i.e. IGHD, ISS and SGA) showed increased HRs. Interestingly, a higher risk of cardiovascular disease was associated with longer duration of rhGH treatment and total cumulative dose (42).

FINAL REMARKS ON CARDIO AND CEREBROVASCULAR RISK IN PATIENTS TREATED WITH rhGH DURING CHILDHOOD

Although GH treatment has been reported to improve cardiovascular risk factors in GHD patients (43), the available data suggest a slight but significant increased cardio and cerebrovascular risk in patients treated with rhGH during childhood (**Table 1**). As mentioned in regard to cancer risk all the reports are burdened by many confounders and biases which prevent from drawing definitive conclusions. It is plausible that GH therapy in association with other risk factors such as genetics, environment, lifestyle, ethnicity, and comorbidities may concur in increasing cardio and cerebrovascular risk.

It has to be pointed out that the association between short stature and cardiovascular risk is well known (44). A genetic approach based on 180 height-associated genetic variants showed that a change in genetically determined height of 1 SD (6.5 cm) was associated with an increase of 13.5% (confidence interval, 5.4 to 22.1) in the risk of coronary artery disease (CAD) (45). In the same study, pathways linking height-associated genes with the risk of CAD were identified. These findings suggest that certain forms of short stature may per se be associated with increased cardio and cerebrovascular risk and the disentanglement of the potential adverse effect of hGH therapy from underlying predisposing factors still remains a major challenge.

DIABETES RISK IN PATIENTS TREATED WITH rhGH DURING CHILDHOOD

The role played by GH in glucose metabolism is well recognized. In particular, the administration of GH decreases glucose uptake and glucose oxidation, increases gluconeogenesis and reduces insulin sensitivity (46, 47).

The first report showing an association between rhGH therapy and risk of diabetes collected data of a large international pharmaco-epidemiological survey for monitoring efficacy and safety of GH therapy in children and adolescents (KIGS) (48). The incidence of type 1 diabetes was not increased in children treated with rhGH whereas the incidence of type 2 diabetes was six-fold higher than expected and diabetes persisted even after discontinuation of rhGH therapy. This finding was confirmed by another multinational observational study of children with growth disorders (GENESIS) which reported a significant higher incidence of type 2 diabetes in children treated with rhGH (49). Risk factors for type 2 diabetes were identified in 10 out of the 11 patients who developed the disease. A further report from GENESIS observational study on a larger population confirmed the increased risk of type 2 diabetes in patients treated with rhGH and with other predisposing factors (27).

In contrast to these findings a French study of the prevalence of diabetes in more than 5000 patients of patients treated with rhGH during childhood, showed no increased risk of diabetes in subjects treated with rhGH (50).

FINAL REMARKS ON DIABETES RISK IN PATIENTS TREATED WITH rhGH DURING CHILDHOOD

The majority of available data suggest that rhGH therapy is associated with increased risk of type 2 diabetes in patients with risk factors such as obesity, genetic predisposition and sedentary lifestyle (**Table 1**). Furthermore, rhGH therapy may function as an accelerator in the development of diabetes in patients with predisposing diseases such as Turner syndrome and organic GHD. Subjects born small for gestational age represent another group of patients potentially at risk of type 2 diabetes and metabolic syndrome, however, to date, the evidence on long-term metabolic safety of rhGH therapy in these subjects is reassuring (51, 52).

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

1. Li CH, Evans HM. The Isolation of Pituitary Growth Hormone. *Science* (1944) 99:183–4. doi: 10.1126/science.99.2566.183
2. Raben MS. Treatment of a Pituitary Dwarf With Human Growth Hormone. *J Clin Endocrinol Metab* (1958) 18:901–3. doi: 10.1210/jcem-18-8-901
3. Prader A, Zachmann M, Poley JR, Illig R, Szeky J. Long-Term Treatment With Human Growth Hormone (Raben) in Small Doses. *Eval 18 Hypopituitary Patients Helv Paediatr Acta* (1967) 22:423–40.
4. Soyka LF, Ziskind A, Crawford JD. Treatment of Short Stature in Children and Adolescents With Human Pituitary Growth Hormone (Raben). *N Engl J Med* (1964) 271:754–64. doi: 10.1056/NEJM196410082711502
5. Koch TK, Berg BO, De Armond SJ, Gravina RF. Creutzfeldt-Jakob Disease in a Young Adult With Idiopathic Hypopituitarism. Possible Relation to the Administration of Cadaveric Human Growth Hormone. *N Engl J Med* (1985) 313:731–3. doi: 10.1056/NEJM198509193131206
6. Centers for Disease C. Fatal Degenerative Neurologic Disease in Patients Who Received Pituitary-Derived Human Growth Hormone. *MMWR Morb Mortal Wkly Rep* (1985) 34:359–60, 365-6.
7. Douet JY, Huor A, Cassard H, Lugan S, Aron N, Mesic C, et al. Prion Strains Associated With Iatrogenic CJD in French and UK Human Growth Hormone Recipients. *Acta Neuropathol Com 9* (2021) 9:145. doi: 10.1186/s40478-021-01247-x
8. Goeddel DV, Heyneker HL, Hozumi T, Arentzen R, Itakura K, Yansura DG, et al. Direct Expression in Escherichia Coli of a DNA Sequence Coding for Human Growth Hormone. *Nature* (1979) 281:544–8. doi: 10.1038/281544a0
9. Ranke MB, Wit JM. Growth Hormone - Past, Present and Future. *Nat Rev Endocrinol* (2018) 14:285–300. doi: 10.1038/nrendo.2018.22
10. Clayton PE, Banerjee I, Murray PG, Renehan AG. Growth Hormone, the Insulin-Like Growth Factor Axis, Insulin and Cancer Risk. *Nat Rev Endocrinol* (2011) 7:11–24. doi: 10.1038/nrendo.2010.171
11. Gallagher EJ, LeRoith D. Minireview: IGF, Insulin, and Cancer. *Endocrinology* (2011) 152:2546–51. doi: 10.1210/en.2011-0231
12. Chesnokova V, Melmed S. Growth Hormone in the Tumor Microenvironment. *Arch Endocrinol Metab* (2019) 63:568–75. doi: 10.20945/2359-3997000000186
13. Boguszewski CL, Boguszewski M. Growth Hormone's Links to Cancer. *Endocr Rev* (2019) 40:558–74. doi: 10.1210/er.2018-00166
14. Steuerman R, Shevah O, Laron Z. Congenital IGF1 Deficiency Tends to Confer Protection Against Post-Natal Development of Malignancies. *Eur J Endocrinol* (2011) 164:485–9. doi: 10.1530/EJE-10-0859
15. Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW, et al. Growth Hormone Receptor Deficiency Is Associated With a Major Reduction in Pro-Aging Signaling, Cancer, and Diabetes in Humans. *Sci Transl Med* (2011) 30:70ra13. doi: 10.1126/scitranslmed.3001845
16. Shevah O, Laron Z. Patients With Congenital Deficiency of IGF-I Seem Protected From the Development of Malignancies: A Preliminary Report. *Growth Horm IGF Res* (2007) 17:54–7. doi: 10.1016/j.ghir.2006.10.007
17. Endo M, Kaneko Y, Shikano T, Minami H, Chino J. Possible Association of Human Growth Hormone Treatment With an Occurrence of Acute Myeloblastic Leukemia With an Inversion of Chromosome 3 in a Child of Pituitary Dwarfism. *Med Pediatr Oncol* (1988) 16:45–7. doi: 10.1002/mpo.2950160111
18. Hara T, Komiyama A, Ono H, Akabane T. Acute Lymphoblastic Leukemia in a Patient With Pituitary Dwarfism Under Treatment With Growth Hormone. *Acta Paediatr Jpn* (1989) 31:73–7. doi: 10.1111/j.1442-200X.1989.tb01272.x
19. Wada E, Murata M, Watanabe S. Acute Lymphoblastic Leukemia Following Treatment With Human Growth Hormone in a Boy With Possible Preanemic Fanconi's Anemia. *Jpn J Clin Oncol* (1989) 19:36–9.
20. Stahnke N. Leukemia in Growth-Hormone-Treated Patients: An Update, 1992. *Horm Res* (1992) 38 Suppl 1:56–62. doi: 10.1159/000182571
21. Bell J, Parker KL, Swinford RD, Hoffman AR, Maneatis T, Lippe B. Long-Term Safety of Recombinant Human Growth Hormone in Children. *J Clin Endocrinol Metab* (2010) 95:167–77. doi: 10.1210/jc.2009-0178
22. Swerdlow AJ, Higgins CD, Adlard P, Preece MA. Risk of Cancer in Patients Treated With Human Pituitary Growth Hormone in the UK, 1959-85: A Cohort Study. *Lancet* (2002) 360:273–7. doi: 10.1016/S0140-6736(02)09519-3
23. Swerdlow AJ, Cooke R, Albertsson-Wikland K, Borgstrom B, Butler G, Cianfarani S, et al. Description of the SAGhE Cohort: A Large European Study of Mortality and Cancer Incidence Risks After Childhood Treatment With Recombinant Growth Hormone. *Horm Res Paediatr* (2015) 84:172–83. doi: 10.1159/000435856
24. Carel JC, Ecosse E, Landier F, Meguellati-Hakkas D, Kaguelidou F, Rey G, et al. Long-Term Mortality After Recombinant Growth Hormone Treatment for Isolated Growth Hormone Deficiency or Childhood Short Stature: Preliminary Report of the French SAGhE Study. *J Clin Endocrinol Metab* (2012) 97:416–25. doi: 10.1210/jc.2011-1995
25. Savendahl L, Maes M, Albertsson-Wikland K, Borgstrom B, Carel JC, Henrard S, et al. Long-Term Mortality and Causes of Death in Isolated GHD, ISS, and SGA Patients Treated With Recombinant Growth Hormone During Childhood in Belgium, The Netherlands, and Sweden: Preliminary Report of 3 Countries Participating in the EU SAGhE Study. *J Clin Endocrinol Metab* (2012) 97:E213–7. doi: 10.1210/jc.2011-2882
26. Deodati A, Ferroli BB, Cianfarani S. Association Between Growth Hormone Therapy and Mortality, Cancer and Cardiovascular Risk: Systematic Review and Meta-Analysis. *Growth Hormone Igf Res* (2014) 24:105–11. doi: 10.1016/j.ghir.2014.02.001
27. Child CJ, Zimmermann AG, Chrousos GP, Cummings E, Deal CL, Hasegawa T, et al. Safety Outcomes During Pediatric GH Therapy: Final Results From the Prospective GeNeSIS Observational Program. *J Clin Endocrinol Metab* (2019) 104:379–89. doi: 10.1210/jc.2018-01189
28. Swerdlow AJ, Cooke R, Beckers D, Borgstrom B, Butler G, Carel JC, et al. Cancer Risks in Patients Treated With Growth Hormone in Childhood: The SAGhE European Cohort Study. *J Clin Endocr Metab* (2017) 102:1661–72. doi: 10.1210/jc.2016-2046
29. Poidvin A, Carel JC, Ecosse E, Levy D, Michon J, Coste J. Increased Risk of Bone Tumors After Growth Hormone Treatment in Childhood: A Population-Based Cohort Study in France. *Cancer Med* (2018) 7:3465–73. doi: 10.1002/cam4.1602
30. Savendahl L, Cooke R, Tidblad A, Beckers D, Butler G, Cianfarani S, et al. Long-Term Mortality After Childhood Growth Hormone Treatment: The SAGhE Cohort Study. *Lancet Diabetes Endocrinol* (2020) 8:683–92. doi: 10.1016/S2213-8587(20)30163-7
31. Allen DB, Backeljauw P, Bidlingmaier M, Biller BMK, Boguszewski M, Burman P, et al. GH Safety Workshop Position Paper: A Critical Appraisal of Recombinant Human GH Therapy in Children and Adults. *Eur J Endocrinol* (2016) 174:P1–9. doi: 10.1530/EJE-15-0873
32. Tedeschi B, Spadoni GL, Sanna ML, Vernole P, Caporossi D, Cianfarani S, et al. Increased Chromosome Fragility in Lymphocytes of Short Normal Children Treated With Recombinant Human Growth Hormone. *Hum Genet* (1993) 91:459–63. doi: 10.1007/BF00217772
33. Cianfarani S, Tedeschi B, Germani D, Prete SP, Rossi P, Vernole P, et al. *In Vitro* Effects of Growth Hormone (GH) and Insulin-Like Growth Factor I and II (IGF-I and -II) on Chromosome Fragility and P53 Protein Expression in Human Lymphocytes. *Eur J Clin Invest* (1998) 28:41–7. doi: 10.1046/j.1365-2362.1998.00247.x
34. Kasuki L, Antunes X, Lamback EB, Gadelha MR. Acromegaly: Update on Management and Long-Term Morbidities. *Endocrinol Metab Clin North Am* (2020) 49:475–86. doi: 10.1016/j.ecl.2020.05.007
35. Wolters TLC, Netea MG, Riksen NP, Hermus A, Netea-Maier RT. Acromegaly, Inflammation and Cardiovascular Disease: A Review. *Rev Endocr Metab Disord* (2020) 21:547–68. doi: 10.1007/s11154-020-09560-x
36. Maffei P, Dassie F, Wennberg A, Parolin M, Vettor R. The Endothelium in Acromegaly. *Front Endocrinol (Lausanne)* (2019) 10:437. doi: 10.3389/fendo.2019.00437
37. van Bunderen CC, van Nieuwpoort IC, van Schoor NM, Deeg DJ, Lips P, Drent ML. The Association of Serum Insulin-Like Growth Factor-I With Mortality, Cardiovascular Disease, and Cancer in the Elderly: A Population-Based Study. *J Clin Endocrinol Metab* (2010) 95:4616–24. doi: 10.1210/jc.2010-0940
38. Xie Y, Huang C, Zhu X, Wang J, Fan X, Fu Z, et al. Association Between Circulating Insulin-Like Growth Factor 1 and Risk of All-Cause and Cause-Specific Mortality. *Eur J Endocrinol* (2021) 185:681–9. doi: 10.1530/EJE-21-0573
39. van Bunderen CC, van Nieuwpoort IC, Arwert LI, Heymans MW, Franken AA, Koppeschaar HP, et al. Does Growth Hormone Replacement Therapy Reduce Mortality in Adults With Growth Hormone Deficiency? Data From

the Dutch National Registry of Growth Hormone Treatment in Adults. *J Clin Endocrinol Metab* (2011) 96:3151–9. doi: 10.1210/jc.2011-1215
40. Gaillard RC, Mattsson AF, Akerblad AC, Bengtsson B, Cara J, Feldt-Rasmussen U, et al. Overall and Cause-Specific Mortality in GH-Deficient Adults on GH Replacement. *Eur J Endocrinol* (2012) 166:1069–77. doi: 10.1530/EJE-11-1028
41. Poidvin A, Touze E, Ecosse E, Landier F, Bejot Y, Giroud M, et al. Growth Hormone Treatment for Childhood Short Stature and Risk of Stroke in Early Adulthood. *Neurology* (2014) 83:780–6. doi: 10.1212/WNL.0000000000000737
42. Tidblad A, Bottai M, Kieler H, Albertsson-Wikland K, Savendahl L. Association of Childhood Growth Hormone Treatment With Long-Term Cardiovascular Morbidity. *JAMA Pediatr* (2021) 175:e205199. doi: 10.1001/jamapediatrics.2020.5199
43. Gazzaruso C, Gola M, Karamouzis I, Giubbini R, Giustina A. Cardiovascular Risk in Adult Patients With Growth Hormone (GH) Deficiency and Following Substitution With GH–An Update. *J Clin Endocrinol Metab* (2014) 99:18–29. doi: 10.1210/jc.2013-2394
44. Rich-Edwards JW, Manson JE, Stampfer MJ, Colditz GA, Willett WC, Rosner B, et al. Height and the Risk of Cardiovascular Disease in Women. *Am J Epidemiol* (1995) 142:909–17. doi: 10.1093/oxfordjournals.aje.a117738
45. Nelson CP, Hamby SE, Saleheen D, Hopewell JC, Zeng L, Assimes TL, et al. Genetically Determined Height and Coronary Artery Disease. *N Engl J Med* (2015) 372:1608–18. doi: 10.1056/NEJMoa1404881
46. Weaver JU, Monson JP, Noonan K, John WG, Edwards A, Evans KA, et al. The Effect of Low Dose Recombinant Human Growth Hormone Replacement on Regional Fat Distribution, Insulin Sensitivity, and Cardiovascular Risk Factors in Hypopituitary Adults. *J Clin Endocrinol Metab* (1995) 80:153–9. doi: 10.1210/jcem.80.1.7829604
47. Moller N, Jorgensen JO. Effects of Growth Hormone on Glucose, Lipid, and Protein Metabolism in Human Subjects. *Endocr Rev* (2009) 30:152–77. doi: 10.1210/er.2008-0027
48. Cutfield WS, Wilton P, Bennmarker H, Albertsson-Wikland K, Chatelain P, Ranke MB, et al. Incidence of Diabetes Mellitus and Impaired Glucose Tolerance in Children and Adolescents Receiving Growth-Hormone Treatment. *Lancet* (2000) 355:610–3. doi: 10.1016/S0140-6736(99)04055-6
49. Child CJ, Zimmermann AG, Scott RS, Cutler GBJr., Battelino T, Blum WF, et al. Prevalence and Incidence of Diabetes Mellitus in GH-Treated Children and Adolescents: Analysis From the GeNeSIS Observational Research Program. *J Clin Endocrinol Metab* (2011) 96:E1025–34. doi: 10.1210/jc.2010-3023
50. Poidvin A, Weill A, Ecosse E, Coste J, Carel JC. Risk of Diabetes Treated in Early Adulthood After Growth Hormone Treatment of Short Stature in Childhood. *J Clin Endocrinol Metab* (2017) 102:1291–8. doi: 10.1210/jc.2016-3145
51. Van Der Steen M, Lem AJ, van der Kaay DCM, Hokken-Koelega ACS. Insulin Sensitivity and Beta-Cell Function in SGA Children Treated With GH and GnRHa: Results of a Long-Term Trial. *J Clin Endocrinol Metab* (2016) 101:705–13. doi: 10.1210/jc.2015-3435
52. van der Steen M, Smeets CCJ, Kerkhof GF, Hokken-Koelega ACS. Metabolic Health of Young Adults Who Were Born Small for Gestational Age and Treated With Growth Hormone, After Cessation of Growth Hormone Treatment: A 5-Year Longitudinal Study. *Lancet Diabetes Endocrinol* (2017) 5:106–16. doi: 10.1016/S2213-8587(16)30422-3

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 03 February 2022
doi: 10.3389/fendo.2022.824315

Brachydactyly Type A3 Is More Commonly Seen in Children With Short Stature But Does Not Affect Their Height Improvement by Growth Hormone Therapy

*Huahong Wu, Yang Li and Hui Li**

Department of Growth and Development, Capital Institute of Pediatrics, Beijing, China

OPEN ACCESS

Edited by:
Gianluca Tornese, Institute for Maternal and Child Health Burlo Garofolo (IRCCS), Italy

Reviewed by:
Gabriela A. Vasques, Universidade de São Paulo, Brazil
Kanetee Busiah, Centre Hospitalier Universitaire Vaudois (CHUV), Switzerland
Fabio Sirchia, University of Pavia, Italy

***Correspondence:**
Hui Li
huiligrowth@163.com

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *01 December 2021*
Accepted: *10 January 2022*
Published: *03 February 2022*

Citation:
Wu H, Li Y and Li H (2022) Brachydactyly Type A3 Is More Commonly Seen in Children With Short Stature But Does Not Affect Their Height Improvement by Growth Hormone Therapy. Front. Endocrinol. 13:824315. doi: 10.3389/fendo.2022.824315

Introduction: To analyze the prevalence of brachydactyly type A3 (BDA3) in children with short stature and the effect on growth hormone (GH) therapy.

Methods: We analyzed the medical records of pediatric patients from July 2009 to July 2021. We included children with short stature defined as their height standard deviation score (HtSDS) < -2 and normal short height as their HtSDS between -2 and -1. We calculated the prevalence of BDA3 in different groups and compared the differences in children's characteristics and the therapeutic effect of GH therapy between the BDA3 and no BDA3 groups.

Results: A total of 752 cases were included. The overall prevalence of BDA3 was 23.1%; with a female predominance (30.8% vs. 16.1%, $P < 0.01$). BDA3 was more prevalent in the short stature group (27.2%) than in the normal short stature group (16.7%) and growth hormone deficiency group (16.5%). Birth length, birth weight, HtSDS, and mid-parental height of children with BDA3 were lower than those without BDA3, but there were no significant differences. In patients with Turner syndrome and idiopathic short stature, the HtSDS of the BDA3 group was significantly lower than that of the no BDA3 group ($P < 0.01$). During four years of GH therapy, the HtSDS improvement per year in the BDA3 group were 0.79 ± 0.29, 0.50 ± 0.31, 0.20 ± 0.30, and 0.10 ± 0.22, which were not significantly different from those in the no BDA3 group. At the end of treatment, there were no significant differences in the duration of treatment and total HtSDS improvement between these two groups.

Conclusions: BDA3 is more commonly seen in children with short stature with a female predominance. BDA3 occurrence is independent of the GH pathway and does not affect the therapeutic effect of GH on short stature children.

Keywords: brachydactyly, short stature, children, growth hormone, height

INTRODUCTION

Brachydactyly type A3 (BDA3) is the most common hand anomaly characterized by a shortened middle phalanx of the fifth finger (1). BDA3 often occurs as an isolated malformation in Chinese children, which can be simply identified by a left-hand wrist X-ray film in pediatric clinics. The prevalence of BDA3 varies significantly among different races, with the highest prevalence in the Asian population and lowest in European and African descents. The prevalence of BDA3 ranged from 8.6%–25.6% in Japanese, 1.0%–19.5% in Native Americans, and 0%–2.1% in European and African descents (2, 3). Notably, Europeans are among the tallest populations worldwide, while Asians are among the shortest (4); thus, we can assume that the prevalence of BDA3 in different populations is inversely related to their average height. The occurrence of BDA3 is related to the disorder of cartilage ossification at the epiphysis and advanced closure of the epiphysis, which is also an essential process in height growth (5). Therefore, BDA3 may be related to height growth. However, the exact mechanism of BDA3 and its association with height growth remain unclear.

In recent years, we have observed an increased incidence of BDA3 during growth and bone age evaluation based on the left-hand wrist X-ray film in pediatric clinics. Most children visited a doctor for short stature (6). For short children with BDA3, there has been no research on whether BDA3 is associated with short stature and whether growth hormone (GH) therapy can effectively improve their height compared to those without BDA3. Therefore, we used real-world data from pediatric clinics to analyze the prevalence of BDA3 in a short stature population, the characteristic differences between children with or without BDA3, and whether GH therapy is effective for short children with BDA3. These findings can provide evidence for understanding the relationship between BDA3 and short stature and can aid in diagnosing and treating children with BDA3 in clinical practice.

MATERIALS AND METHODS

Data Resource

Medical records were collected retrospectively from the Growth and Development Clinic of the Capital Institute of Pediatrics, Beijing, China, from July 2009 to July 2021. Considering that the physical growth and development level of children with precocious puberty or advanced development was inconsistent with their chronological age, which may affect the evaluation of children's actual height level, we excluded children diagnosed with precocious puberty and advanced development with normal height in this study. Therefore, only complete medical records with physical measurements, a left-hand wrist X-ray film, definite diagnosis, regular follow-up, therapy information, and those with short stature or normal shorter height were included. Meanwhile, we required a left-hand wrist X-ray film that would allow precise identification of the presence of BDA3. The diagnosis of BDA3 for all patients was strictly assigned following the diagnostic criteria.

Sample Screen and Division

The height and weight recorded in medical cases were measured by trained staff or nurses. We calculated the standard deviation score of patients' height (HtSDS) and weight (WtSDS) using Chinese children's growth references (7). In this study, an HtSDS of < -1 was defined as normal short height; therefore, cases with HtSDS of ≥ 1, usually with advanced bone age, were excluded. We divided all included cases into two groups: those with an HtSDS < -2 as the short stature group and those with an HtSDS between -2 and -1 as the normal short group. The screening procedure is shown in **Figure 1**.

Definition of BDA3

Figure 2A shows the normal left hand-wrist X-ray file without BDA3. The definition of BDA3 varies among different studies. Therefore, we chose a relatively objective definition (8), wherein BDA3 was considered when the middle phalanx of the fifth finger was shorter than half of the middle phalanx of the fourth finger (**Figure 2B**). All cases met this standard regardless of a curved middle phalanx of the fifth finger to the radial side (**Figure 2C**), or conical epiphysis (**Figure 2D**) were classified as the BDA3 group; whereas those who did not satisfy the criteria were classified as the no BDA3 group. We performed the x-ray for the purpose of diagnosing BDA3, and bone age was not analyzed in the study participants. If several left-hand wrist X-ray film were performed, we only selected their first X-ray film to analyze.

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0(IBM, NY, USA). Continuous variables were described as mean ± standard deviation (SD), and the differences between the BDA3 and no BDA3 groups were tested using the t-test. The categorical variables were described by frequency and percentage n (%), and the differences between different groups were tested using the χ^2 test. Statistical significance was set at $P < 0.05$. The effects of GH therapy were calculated based on the changes in children's HtSDS (ΔHtSDS) during treatment.

RESULTS

A total of 752 children with short stature or normal short stature were included in this survey. We included a total of 463 cases of short stature children, wherein 279 had idiopathic short stature (ISS), 91 with growth hormone deficiency (GHD), 42 with Turner syndrome (TS), 27 with small for gestational age (SGA), and 24 had other conditions (including 11 cases of hypothyroidism, 8 cases of preterm infants, 3 cases of Noonan syndrome, 1 case of Laron syndrome, and 1 case of DiGeorge syndrome). The other 288 patients were in the normal short group (**Table 1**).

Among 752 patients, there were 392 boys and 360 girls in this study. The overall ratio of boys to girls was 1.08:1. In case of the GHD, ISS, SGA, and normal short stature groups, the ratios of boys to girls were 2.92:1, 1.06:1, 1.05:1, and 0.93:1, respectively. The age and HtSDS distributions of all cases are

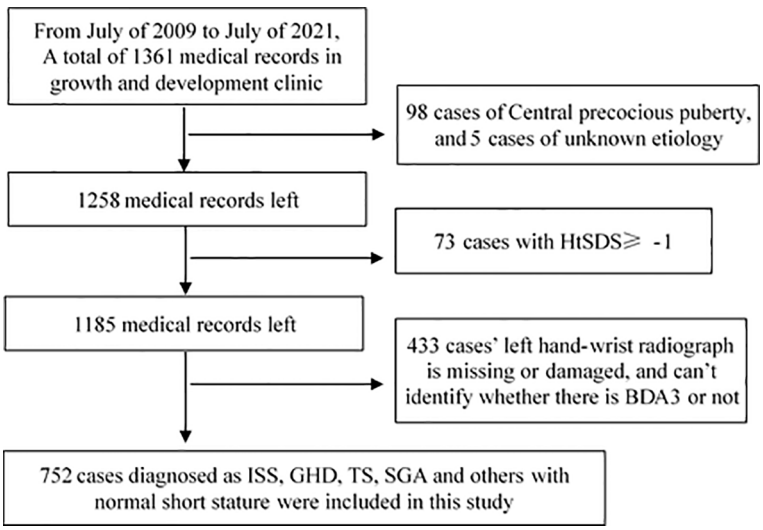


FIGURE 1 | Screening procedure of survey samples.

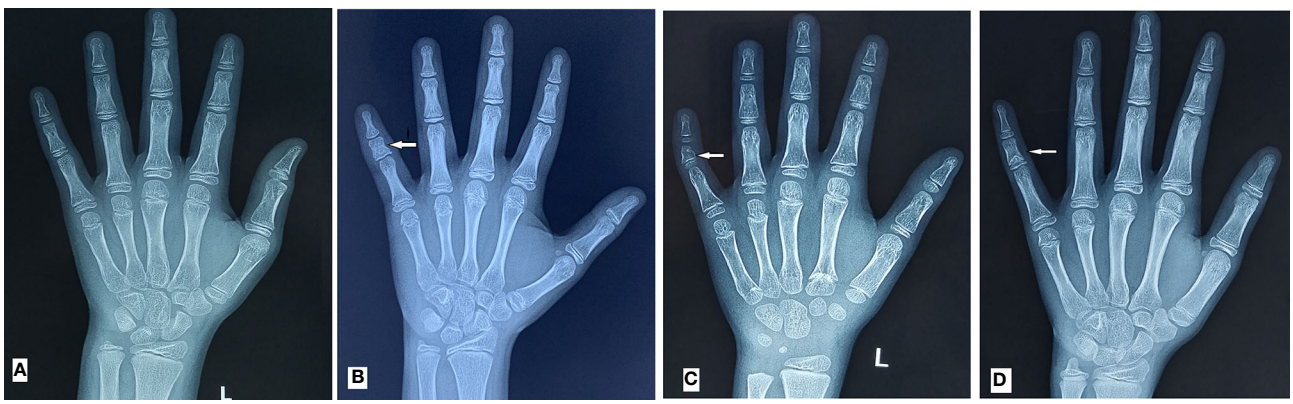


FIGURE 2 | BDA3 diagnostic diagram. **(A)** is the normal left hand-wrist X-ray film; **(B)** is the middle phalanx of fifth finger shorter than half of the middle phalanx of fourth finger; **(C)** is the shorter middle phalanx of fifth finger with curved middle phalanx of fifth finger to the radial side; **(D)** is the shorter middle phalanx of fifth finger with conical epiphysis; BCD were all diagnosed as BDA3.

shown in **Figure 3**. The mean age is 8.8 ± 3.1 years (range 0.3–16.9 years), and the HtSDS is -2.56 ± 1.24 (range -9.83 to -1.01).

Prevalence of BDA3

The overall prevalence of BDA3 was 23.1% (174/752), with a higher prevalence in girls at 30.8% (111/360) compared to boys at 16.1% (63/392) ($P < 0.01$). Even excluded those girls with Turner syndrome, the prevalence of BDA3 in girls (29.2%) is still significantly higher than that in boys (16.1%) ($P < 0.01$).

We further analyzed the prevalence of BDA3 in the normal short stature and short stature groups with different etiologies. **Table 1** shows that the prevalence of BDA3 in the short stature group was 27.2%, which was significantly higher than that in the normal short group (16.7%). In the short stature group, the prevalence of BDA3 in patients with TS and SGA were exceed 40%. The prevalence of BDA3 in patients with GHD was lower than that in the normal short group. There was only one case of BDA3 in hypothyroidism and preterm patients. There were significant differences in the prevalence of BDA3 among the different groups.

Comparison of Children's Characteristics Between the BDA3 and No BDA3 Group

Table 2 shows the general information, birth size, growth level, and mid-parental height of children in the BDA3 and no BDA3 groups. The chronological age and bone age of children in the BDA3 group were smaller than those in the no BDA3 group, but the difference between the chronological age and bone age (BAD)

TABLE 1 | The prevalence of BDA3 in different groups [n (%)].

	N	BDA3		χ^2	*p*
		NO	YES		
Normal short	288	240 (83.3)	48 (16.7)	11.096	0.001
Short stature	463	337 (72.8)	126 (27.2)		
ISS	279	199 (71.3)	80 (28.7)	17.841	0.007
GHD	91	76 (83.5)	15 (16.5)		
TS	42	24 (57.1)	18 (42.9)		
SGA	27	16 (59.3)	11 (40.7)		
Others	24	22 (91.7)	2 (8.3)		

BDA3, brachydactyly type A3; ISS, idiopathic short stature; GHD, growth hormone deficiency; TS, Turner syndrome; SGA, small for gestational age.

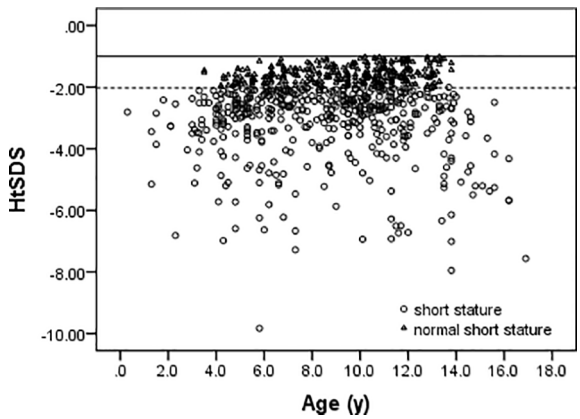


FIGURE 3 | The age and HtSDS distribution of all cases.

in these two groups was similar. This showed that BDA3 may not be related to the process of skeletal maturation and bone aging. Meanwhile, we observed that the birth weight, birth length, HtSDS, and WtSDS of the BDA3 group were slightly smaller than those in the no BDA3 group, but there were no significant differences ($P > 0.05$) (**Table 2**). In addition, the mid-parental height in the BDA3 group was also shorter than that in the no BDA3 group, which was significantly different in short stature children.

The short stature group consisted of patients with different etiologies; therefore, we further compared the HtSDS of children with different diagnoses to analyze whether patients with BDA3 were shorter than those without BDA3. **Figure 4** shows that in all short stature patients, there was a tendency of the HtSDS of children with BDA3 slightly lower than those without BDA3, but the differences were statistically significant only in TS and ISS patients. In patients with GHD and SGA, there were no significant differences in HtSDS between the BDA3 and no BDA3 groups.

Association of BDA3 and the Therapeutic Effect of GH on Short Stature Children

Among the 463 short stature children in this survey, 346 received GH treatment for longer than one year. However, patients diagnosed with GHD, TS, SGA, and premature birth have confounding factors, such as growth hormone deficiency, chromosomal abnormality, and fetal abnormality, which render the analysis of the impact of BDA3 on the therapeutic effect of GH impossible. Therefore, we only analyzed whether BDA3 affected the therapeutic effect of GH in children with ISS without other confounding factors.

Table 3 shows the age, height, and GH therapeutic effect of ISS patients with BDA3. ISS patients in the BDA3 group were younger and shorter than those in the no BDA3 group, but there was no significant difference in their HtSDS between these two groups. During the four years of GH therapy, the therapeutic effect (ΔHtSDS) decreased annually, and there were no significant differences in each year's ΔHtSDS between the BDA3 and no BDA3 groups. At the end of treatment, there were still no significant differences in the duration of treatment and total ΔHtSDS between the two groups.

DISCUSSIONS

We analyzed the association of BDA3 with short stature in a pediatric population. We also investigated the prevalence of BDA3, response to GH therapy in short stature children with

TABLE 2 | The comparison of children's characteristics between BDA3 and no BDA3 group (Mean ± SD).

	BDA3		*Difference (95%CI)*	*t*	*p*
	NO	**YES**			
Normal short group					
N	240	48			
Chronology age (y)	9.5 ± 2.5	8.9 ± 2.7	0.72 (-0.06,1.51)	1.813	0.071
Bone Age (y)	9.6 ± 2.6	8.5 ± 2.8	1.09 (0.02,2.16)	2.007	0.047
BAD (y)	0.2 ± 0.9	0.3 ± 0.9	-0.04 (-0.34,0.28)	-0.233	0.816
Birth Weight (kg)	3.14 ± 0.52	3.11 ± 0.46	0.03 (-0.14,0.19)	0.356	0.772
Birth Length (cm)	49.4 ± 2.1	48.9 ± 2.4	0.44 (-0.45,1.33)	0.983	0.327
HtSDS	-1.54 ± 0.26	-1.60 ± 0.29	0.05 (-0.03,0.14)	1.325	0.186
WtSDS	-1.00 ± 0.80	-1.15 ± 1.06	0.16 (-0.11,0.42)	1.151	0.251
Mid-Parent Height (cm)	164.0 ± 7.2	161.7 ± 7.3	2.27 (-0.06,4.60)	1.919	0.056
Short stature group					
N	337	126			
Chronology age (y)	8.5 ± 3.5	8.0 ± 3.4	0.48 (-0.21,1.19)	1.355	0.176
Bone Age (y)	7.8 ± 3.3	7.1 ± 3.4	0.76 (-1.33,1.64)	1.674	0.095
BAD (y)	1.4 ± 1.7	1.1 ± 1.4	0.26 (-0.11,0.63)	1.385	0.167
Birth Weight (kg)	3.06 ± 0.53	2.98 ± 0.48	0.08 (-0.04,0.19)	1.355	0.176
Birth Length (cm)	49.3 ± 2.1	48.8 ± 1.9	0.57 (-0.10,1.25)	1.672	0.096
HtSDS	-3.17 ± 1.23	-3.22 ± 1.11	0.05 (-0.20,0.30)	0.396	0.692
WtSDS	-1.99 ± 0.94	-2.08 ± 0.99	0.09 (-0.11,0.29)	0.897	0.370
Mid-Parent Height (cm)	163.6 ± 7.7	160.7 ± 7.2	2.9 (1.3,4.5)	3.607	0.000

BDA3, brachydactyly type A3; BAD, Chronology age- Bone Age; HtSDS, height standard deviation score; WtSDS, weight standard deviation score; Mid-Parent Height, (father height + mother height ±13cm)/2.

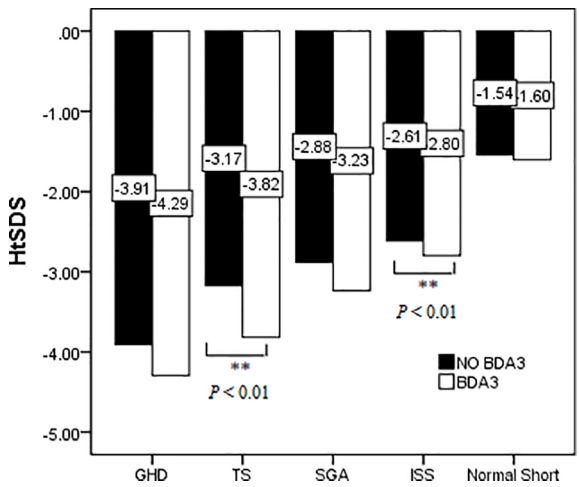


FIGURE 4 | Effect of BDA3 in children's HtSDS with different etiologies. ** means that there is a significant difference p < 0.01.

and without BDA3, and characteristic differences between children with and without BDA3. The prevalence of BDA3 in short stature children is higher than that in normal height children, with a female predominance. BDA3 did not affect the effect of GH therapy on children with BDA3.

Because left-hand wrist radiography carries a risk of radioactive exposure, it is difficult to perform large-scale surveys using this modality in the general population, so there are almost no studies on the prevalence of BDA3 in China. In this survey, we found that the total prevalence of BDA3 in short stature and normal short children was 23.1%, which is significantly higher than the 6.95% in a relatively normal population survey in China (8), and was also higher than that of Chinese descents in the United States (12.5%) and a previous small sample survey in China conducted in 1967 (5.0%) (9, 10). Meanwhile, the prevalence of BDA3 in the short stature group was 27.2%, which was also significantly higher than that in the normal short group (16.7%). These results revealed that BDA3 is more commonly seen in children with short stature. In addition, BDA3 is more commonly seen in girls than in boys, which is consistent with the conclusions of other Asian populations (11). In a series of surveys on the Japanese population from 1942 to

TABLE 3 | The therapeutic effects of GH on ISS patients in BDA3 and no BDA3 group (mean ± SD).

	BDA3		*t*	*p*
	NO	YES		
Start of therapy				
N	153	55		
Age(y)	8.4 ± 3.3	8.0 ± 3.4	0.747	0.456
Height(cm)	118.2 ± 16.1	114.9 ± 17.4	1.290	0.199
Mid-Parent Height (cm)	162.7 ± 7.9	160.1 ± 8.0	1.990	0.048
HtSDS	-2.54 ± 0.56	-2.70 ± 0.69	1.690	0.092
GH dose(IU/kg/d)	015 ± 0.02	0.16 ± 0.02	-1.928	0.055
After 1st year of therapy				
N	147	48		
Height(cm)	127.0 ± 16.0	123.8 ± 17.7	1.174	0.242
HtSDS	-1.78 ± 0.59	-1.96 ± 0.81	1.669	0.097
$\Delta HtSDS_{1st}$	0.77 ± 0.39	0.79 ± 0.29	-0.292	0.771
After 2nd year of therapy				
N	96	34		
Height(cm)	135.0 ± 15.1	128.3 ± 15.3	2.053	0.042
HtSDS	-1.35 ± 0.69	-1.55 ± 0.89	1.371	0.173
$\Delta HtSDS_{2nd}$	0.45 ± 0.31	0.50 ± 0.31	-0.823	0.412
After 3rd year of therapy				
N	40	19		
Height(cm)	140.6 ± 12.6	128.1 ± 17.9	3.002	0.004
HtSDS	-1.00 ± 0.67	-1.30 ± 0.90	1.458	0.150
$\Delta HtSDS_{3rd}$	0.28 ± 0.22	0.20 ± 0.30	1.167	0.248
After 4th years of therapy				
N	23	8		
Height(cm)	144.7 ± 13.7	125.3 ± 3.7	3.928	0.001
HtSDS	-0.90 ± 0.59	-1.02 ± 0.52	0.507	0.616
$\Delta HtSDS_{4th}$	0.23 ± 0.34	0.10 ± 0.22	1.064	0.297
The end of therapy				
N	153	55		
Age(y)	11.1 ± 3.3	10.8 ± 3.3	0.511	0.610
GH therapy time(m)	28.5 ± 16.4	29.3 ± 19.2	-0.293	0.770
Height(cm)	138.3 ± 17.4	135.6 ± 15.6	1.019	0.310
HtSDS	-1.25 ± 0.76	-1.39 ± 0.77	1.168	0.244
$\Delta HtSDS_{total}$	1.29 ± 0.69	1.31 ± 0.68	-0.212	0.832

Mid-Parent Height, (father height + mother height ±13cm)/2; HtSDS, height standard deviation score; ΔHtSDS, the changes in children's HtSDS during treatment.

1973 and one survey on the Japanese descents in the United States, a female predominance was likewise noted (2, 12). In other surveys in Native Americans, Mexico, and Pacific island countries, BDA3 is also more frequently seen in females (12–14). However, this phenomenon was not observed in Caucasians (15). The reason why BDA3 is more common in short children and girls needs to be explained by its exact mechanism.

The mechanism and pathogenic gene of BDA3 remain unclear. This may be a combination of complicated mechanisms involving multiple genes and pathogenic pathways. In recent years, it has been found that BDA3 may be an autosomal dominant condition with an obvious familial genetic tendency (1). A Chinese study in 2020 stated that the deletion of the *HOXD13* gene is related to the occurrence of familial BDA3 and BDA4 (16). Vasques et al. reported that BDA3 was observed in 64.3% of hand radiographs from individuals heterozygous from *IHH* variants initially classified as ISS (17). However, Williams et al. did not support the autosomal dominant inheritance model of BDA3 (2). In this study, we found that the prevalence of BDA3 in children with chromosomal abnormalities such as TS was 42.9%, suggesting that the deletion of specific spots on the X chromosome may be related to the occurrence of BDA3. Otherwise, BDA3 may occur in the fetus and is related to the abnormal development of the fetus during the first nine weeks of pregnancy (18), which directly affects the birth weight and body length of the fetus. The prevalence of more than 40% of BDA3 in SGA children in this study also indirectly confirms the above conclusion. However, some children with BDA3 grow normally during fetal development (delivered with a normal birth length according to standardized growth charts) but later exhibit short stature as they age, as seen in the patients with ISS. Therefore, the prevalence of BDA3 in children with ISS is also higher than in normal children, and this often results in a shorter adult height (18). In addition, BDA3 can appear in some syndromes such as Silver Russell syndrome, Coffin-Siris syndrome, Down syndrome (5, 19), and a pair of identical twin girls (8). Further research needs to confirm whether the specific pathogenesis of BDA3 involves autosomal, sex chromosomes, or multiple etiologies. We only know that the prevalence of BDA3 in GHD patients is relatively low, even lower than that in the normal short group, which may imply that the occurrence of BDA3 is independent of GH secretion.

BDA3 did not affect the therapeutic effect of GH on short-stature children. **Table 3** shows no significant differences in ΔHtSDS between the BDA3 and no BDA3 groups during the first, second, third, and fourth years of GH therapy. At the end of the therapy, there were no differences in HtSDS and total ΔHtSDS between the two groups. This further confirmed that BDA3 has nothing to do with the GH pathway, and short stature children with simple BDA3 can also improve their height by GH therapy similar to those without BDA3. Pereda and Vasques have confirmed that GH therapy can improve the height of children with short stature with other types of brachydactyly, but their study had no normal control group (6, 17, 20). Besides, our study suggests that BDA3 was not related to the process of skeletal maturation, bone aging, and the degree of short stature, which may be due to the relatively small sample of children in this study. So further longitude study is still needed to explore the relationship between BDA3, skeletal maturation process, and adult short stature.

So far, this is the first study on the effect of BDA3 on the therapeutic effects of GH in short stature children. The strength of this study is the longitudinal follow-up data of GH therapy on short stature children with BDA3. However, our study also has some limitations. First, in this study, medical records from July 2009 to July 2021 were retrospectively analyzed. Before 2015, the clinical application of second-generation sequencing technology was very limited. Therefore, some short stature children with bone abnormalities or bone genetic disorder were not definitely diagnosed with these conditions. After 2015, almost all short stature children with multiple skeletal deformities were evaluated by a geneticist. However, in this study, we only included children with isolated brachydactyly type A3 (BDA3) and not those with syndromic forms of BDA3. Second, cross-sectional data were collected from a single pediatric clinic, so the sample is relatively small and cannot represent all kinds of short-stature children. Therefore, whether BDA3 can aggravate short stature in children still needs to be verified using larger sample surveys. Third, this study did not include genetic characteristics and whole family spectrum analyses of BDA3 patients, so we cannot distinguish whether BDA3 is hereditary or spontaneous, and weather different kind of BDA3 has different effect on children's height growth and their therapeutic effect of GH.

CONCLUSIONS

In conclusion, BDA3 is more commonly seen in short stature children than in normal height populations and manifests predominantly in girls. BDA3 occurrence is independent of the GH pathway and does not affect the therapeutic effect of GH on short-stature children. However, the mechanisms and pathogenic genes of BDA3 are not clear. Therefore, further research should include genetic characteristics and whole family spectrum analyses of BDA3 patients to explore the inheritance and pathogenesis mechanism of this disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Review Committee of the Capital Institute of Pediatrics. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

HW was involved in data collecting and review, in charge of this paper's results interpretation and paper writing. HL was in charge of survey design, data review supervision, results interpretation, and paper writing. YL was involved in data collecting and review. All authors agreed with the data interpretation and approved the final version of the manuscript.

FUNDING

This study was supported by The Special Fund of the Pediatric Medical Coordinated Development Center of Beijing Hospitals Authority (XTZD20180403), Public service development and reform pilot project of Beijing Medical Research Institute (BMR2019-11), and CAMS Innovation Fund for Medical Sciences (CIFMS) (2016-I2M-1-008).

ACKNOWLEDGMENTS

We greatly appreciate all patients and their parents included in this survey and other participants in physical examination, bone age film shooting, and medical cases management.

REFERENCES

1. Temtamy SA, Aglan MS. Brachydactyly. *Orphanet J Rare Dis* (2008) 3:15. doi: 10.1186/1750-1172-3-15
2. Williams KD, Blangero J, Cottom CR, Lawrence S, Choh AC, Czerwinski SA, et al. Heritability of Brachydactyly Type A3 in Children, Adolescents, and Young Adults From an Endogamous Population in Eastern Nepal. *Hum Biol* (2007) 79:609–22. doi: 10.1353/hub.2008.0016
3. Williams KD, Nahhas RW, Cottom CR, Lawrence S, Subedi J, Jha B, et al. Evaluation of Qualitative Methods for Phenotyping Brachymesophalangia-V From Radiographs of Children. *Am J Hum Biol* (2012) 24:68–73. doi: 10.1002/ajhb.22205
4. Rodriguez-Martinez A, Zhou B, Sophiea MK, Bentham J, Paciorek CJ, Iurilli ML, et al. Height and Body-Mass Index Trajectories of School-Aged Children and Adolescents From 1985 to 2019 in 200 Countries and Territories: A Pooled Analysis of 2181 Population-Based Studies With 65 Million Participants. *Lancet* (2020) 396:1511–24. doi: 10.1016/S0140-6736(20)31859-6

5. Yang X. Etiology, Classification and Treatment of Brachydatyly J. *J Tissue Eng Reconstr Surg* (2015) 11:389–95. doi: 10.3969/j.issn.1673-0364.2016.06.012
6. Xuyun H, Di W, Mengtin L, Jiajia C, Xiaoqiao L, Chang S, et al. Gene Mutations and Clinical Phenotypes in Three Families With Short Stature and Brachydactyly and Review of Literature. *J Capital Med Univ* (2018) 39:937–44. doi: 10.3969/j.issn.1006-7795.2018.06.025
7. Li H, Ji CY, Zong XN, Zhang YQ. Height and Weight Standardized Growth Charts for Chinese Children and Adolescents Aged 0 to 18 Years. *Zhonghua Er Ke Za Zhi* (2009) 47:487–92. doi: 10.3760/cma.j.issn.n0578-1310.2009.07.003
8. Shan Y, Baosheng Y, Lanying G, Anru W. Detection Rate of Brachydachydactyly Type A3 in 1208 Han Chinese Children. *Chin J Appl Clin Pediatr* (2018) 33:1586–7. doi: 10.3760/cma.j.issn.2095-428X.2018.20.016
9. Hertzog K. Shortened Fifth Medial Phalanges. *Am J Phys Anthropol* (1967) 27:113–8. doi: 10.1002/ajpa.1330270202
10. Garn SM, Fels SL, Israel H. Brachymesophalangia of Digit Five in Ten Populations. *Am J Phys Anthropol* (1967) 27:205–10. doi: 10.1002/ajpa.1330270208
11. Takatama H, Minooka M. Studies of the Brachymesophalangia of the Little Finger in the Ainu and Japanese Schoolchildren in Niikappu, Hokkaido Sapporo. *Med J* (1976) 45:166–76. doi: 10.15114/smj.45.166
12. Greulich W. The Incidence of Dysplasia of the Middle Phalanx of the Fifth Finger in Normal Japanese, in Some American Indian Groups, and in Caucasians With Down's Syndrome. In: N Kretchmer, DN Walcher, editors. *Environmental Influences on Genetic Expression: Biological and Behavioral Aspects of Sexual Differentiation*. Bethesda, MD: National Institutes of Health (1970). p. 91–105.
13. Brown T, Lambert W, Pinkerton SK. Brachymesophalangia-5 in a Group of Australian Aboriginals. *Hum Biol* (1980) 52:651–9.
14. Abbie AA. Brachymesophalangy V in Australian Aborigines. *Med J Aust* (1970) 2:736–7. doi: 10.5694/j.1326-5377.1970.tb63148.x
15. Buschang PH, Malina RM. Brachymesophalangia-V in Five Samples of Children: A Descriptive and Methodological Study. *Am J Phys Anthropol* (1980) 53:189–95. doi: 10.1002/ajpa.1330530203
16. Zhang M, Lu L, Wei B, Zhang Y, Li X, Shi Y, et al. Brachydactyly Type A3 is Caused by a Novel 13 Bp HOXD13 Frameshift Deletion in a Chinese Family. *Am J Med Genet A* (2020) 182:2432–6. doi: 10.1002/ajmg.a.61788
17. Vasques GA, Funari MFA, Ferreira FM, Aza-Carmona M, Sentchordi-Montané L, Barraza-García J, et al. IHH Gene Mutations Causing Short Stature With Nonspecific Skeletal Abnormalities and Response to Growth Hormone Therapy. *J Clin Endocrinol Metab* (2018) 103:604–14. doi: 10.1210/jc.2017-02026
18. Garn SM, Babler WJ, Burdi AR. Prenatal Origin of Brachymesophalangia-5. *Am J Phys Anthropol* (1976) 44:413–6. doi: 10.1002/ajpa.1330440305
19. Garn SM, Gall JCJr, Nagy JM. Brachymesophalangia-5 Without Cone-Epiphysis Mid-5 in Down's Syndrome. *Am J Phys Anthropol* (1972) 36:253–5. doi: 10.1002/ajpa.1330360213
20. Pereda A, Garzon-Lorenzo L, Garin I, Cruz-Rojo J, Sanchez Del Pozo J, Perez de Nanclares G. The P.R56* Mutation in PTHLH Causes Variable Brachydactyly Type E. *Am J Med Genet A* (2017) 173:816–9. doi: 10.1002/ajmg.a.38067

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 16 February 2022
doi: 10.3389/fendo.2022.820001

Clinical Characteristics of Short-Stature Patients With Collagen Gene Mutation and the Therapeutic Response to rhGH

*Meiping Chen, Hui Miao, Hanting Liang, Xiaoan Ke, Hongbo Yang, Fengying Gong, Linjie Wang, Lian Duan, Shi Chen, Hui Pan and Huijuan Zhu**

Key Laboratory of Endocrinology of National Health Commission, Department of Endocrinology, State Key Laboratory of Complex Severe and Rare Diseases Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

OPEN ACCESS

Edited by:
Robert Rapaport, Icahn School of Medicine at Mount Sinai, United States

Reviewed by:
Dimitra Micha, Amsterdam University Medical Center, Netherlands
Ke Yuan, Zhejiang University, China
Cassie Mintz, Icahn School of Medicine at Mount Sinai, United States

***Correspondence:**
Huijuan Zhu
shengxin2004@163.com

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *22 November 2021*
Accepted: *06 January 2022*
Published: *16 February 2022*

Citation:
Chen M, Miao H, Liang H, Ke X, Yang H, Gong F, Wang L, Duan L, Chen S, Pan H and Zhu H (2022) Clinical Characteristics of Short-Stature Patients With Collagen Gene Mutation and the Therapeutic Response to rhGH. Front. Endocrinol. 13:820001. doi: 10.3389/fendo.2022.820001

Context: Clinical genetic evaluation has been demonstrated as an important tool to elucidate the causes of growth disorders. Genetic defects of collagen formation (the collagenopathies) have been reported to be associated with short stature and skeletal dysplasias. Etiological diagnosis of skeletal abnormality-related short stature is challenging, and less is known about recombinant human growth hormone (rhGH) therapy.

Objective: This is a single-center cohort study which aims at exploring the genetic architecture of short-stature children with skeletal abnormalities and evaluating the frequency of collagenopathies to determine their phenotype, including the rhGH treatment response.

Patients and Methods: One hundred and six children with short stature and skeletal abnormalities were enrolled who were evaluated by next-generation sequencing (NGS) to detect variants in the skeletal collagen genes including *COL1A1, COL1A2, COL2A1, COL9A1, COL9A2, COL9A3, COL10A1, COL11A1*, and *COL11A2*. The results were evaluated using American College of Medical Genetics and Genomics (ACMG) guidelines. Clinical characteristics and rhGH treatment response were summarized.

Results: Twenty-four pathogenic or likely pathogenic variants of collagen genes were found in 26 of 106 (24.5%) short-stature patients with skeletal abnormalities, of which *COL2A1* mutations were the most common, accounting for about 57.7%. Other frequent mutations associated with skeletal development include *FGFR3*, *ACAN*, *NPR2*, *COMP*, and *FBN1* in 12.2%, 0.9%, 0.8%, 0.4%, and 0.4%, respectively, resulting in significantly different degrees of short stature. An overview of clinical features of collagenopathies showed growth retardation, skeletal abnormalities, and heterogeneous syndromic abnormalities involving facial, eye, hearing, and cardiac abnormalities. The average height of 9 patients who received rhGH treatment improved from a median of -3.2 ± 0.9 SDS to -2.2 ± 1.3 SDS after 2.8 ± 2.1 years. The most significant height improvement

of 2.3 SDS and 1.7 SDS was also seen in two patients who had been treated for more than 6 years.

Conclusions: A proband-based NGS revealed that distinct genetic architecture underlies short stature in varying degrees and clinical features. Skeletal abnormality-related short stature involving multiple systems should be tested for skeletal collagen gene mutation. Limited rhGH treatment data indicate an improved growth rate and height, and close monitoring of adverse reactions such as scoliosis is required.

Keywords: short stature, skeletal abnormalities, collagenopathies, next-generation sequencing, growth hormone treatment

INTRODUCTION

Childhood linear growth is the result of chondrogenesis at the skeletal growth plate, the structure responsible for bone elongation and therefore overall body size (1). Recently, findings have uncovered a vast array of regulatory systems that implicate multiple aspects of the growth plate and long bone development and an accompanying vast array of genetic defects that can cause disorders of linear growth (2). Some sequence variations in genes affecting growth plate function can produce a phenotypic spectrum of short stature with skeletal dysplasia, ranging from severe skeletal deformity to disproportionately short stature, most of which show severe short stature (2). The etiological diagnosis for short stature with skeletal abnormalities is still a clinical challenge, and therapy for improving their severe short stature has been rarely attempted.

With the advances of broad sequencing approaches, clinical genetic evaluation has been demonstrated as an important tool to elucidate the causes of growth disorders from among the myriad possibilities, and an increasing number of short stature-associated genes have been discovered. These causative genes are involved in the physiological processes of the growth plate and long bone development, including normal production and action of multiple hormones, paracrine signaling, and extracellular matrix (ECM) molecules (e.g., cartilage oligomeric matrix protein, aggrecan, several different types of collagens produced by chondrocytes), as well as the normal function of multiple intracellular processes required for chondrocyte proliferation, hypertrophy, and extracellular matrix production (2, 3). Some typical genetic syndromes have been identified, such as Laron syndrome (MIM #262500) related to *GHR*, Leri–Weill dyschondrosteosis (MIM #127300) related to *SHOX*, Noonan syndrome (MIM #163950), and Silver–Russell syndrome (MIM #180860), which are associated with short stature and various multiorgan malformations (4–6). Genetic disorders associated with skeletal dysplasia include many genes involved in growth plate development, such as *FGFR3*, *ACAN*, *NPR2*, *FBN1*, and *IHH*, which can cause varying degrees of short stature with or without other minor abnormalities (7, 8). Recently, not only for *ACAN* (aggrecan) and *COMP* (cartilage oligomeric matrix protein) in ECM components but also for *NPR2* (natriuretic peptide receptor 2) in paracrine signaling, we reported the phenotypic and genotypic spectra and efficacy of GH therapy for height gain. For collagen, the most abundant protein in the human body, however, the prevalence of collagen gene mutation in short-stature patients with skeletal abnormalities is yet unknown, the current clinical manifestations of the disease are heterogeneous, and the response data of growth hormone therapy are limited. Collagen types II, IX, X, and XI are present in a growth plate important to long bone development and joint health, and type I collagen is the primary collagen in bone for bone formation, growth, and remodeling, and subsequently mineralization to form bone tissue. Mutations in genes that encode skeletal collagen are not uncommon in the genetic causes for growth defects with skeletal abnormalities (9).

Subsequently, we analyzed 106 children with short stature by using a gene panel for short stature and whole-exome sequencing (WES) from our single-center cohort and searched for variants in the skeletal collagen genes. Variant interpretation, genotype–phenotype analyses, and the response to rhGH treatment of skeletal abnormality-related short stature were investigated.

PATIENTS AND METHODS

Patients

One hundred and six children with short stature and skeletal abnormalities in our endocrinology department were included, 64 of whom received WES and 42 of whom received a short stature-targeted gene panel sequencing. Skeletal abnormalities were characterized by an intrinsic abnormality in growth and (re-)modeling of cartilage and bone, including the whole-body skeleton of axial bones, limbs, and craniofacial bones, which were assessed by a professional physician through physical examination and measurements. All probands fulfilled the following diagnostic criteria: height standard deviation (SD) ≤ -2 with skeletal abnormalities, absence of abnormal findings on clinical examination or in laboratory tests that could account for short statures, such as hypothyroidism and GHD, and known Noonan syndrome and Turner syndrome. Clinical materials of the first and follow-up visits of the probands, including history-taking, physical examination, and auxiliary examination, were collected. Information about rhGH therapy was also reviewed and recorded. Peripheral blood samples of patients and their available relatives were collected, and genomic DNA was obtained from peripheral blood leukocytes by using

standard techniques. The patients or guardians signed informed consent forms regarding the research, and this study was performed with the approval of the Ethics Committee of Peking Union Medical College Hospital.

Whole-Exome Sequencing

The 3-μg genomic DNA concentrations were sheared with a Covaris LE220 ultrasonic instrument (MA, USA) to a target of 100–500-bp average size. Then, the DNA fragments with a main fragment size of 150–200 bp were screened by magnetic beads to create a DNA library for each subject. The library was qualitatively controlled by Agilent 2100 Bioanalyzer (BGI, Shenzhen, China). All amplified libraries were subsequently sent to BGI for circularization and sequencing on the BGISEQ-500 platform, and the primary sequencing data were read out. To detect the potential variants in the family, bioinformatics processing and data analysis were performed after receiving the primary sequencing data. Sequencing data were aligned to the human genome reference (hg19) using the BWA (Burrows–Wheeler Aligner) Multi-Vision software package to analyze single-nucleotide variants (SNVs) and INDEL calling (10). All SNVs and indels were filtered and estimated *via* multiple databases, including NCBI dbSNP, HapMap, 1000 human genome dataset, and database of 100 Chinese healthy adults.

Targeted Sequencing

A capture panel (NimbleGen, Madison, USA) of short-stature genes was previously designed and assessed by our group. The capture panel covered all exons together with the flanking exon and intron boundaries (± 15 bp) of 466 genes. Sequencing was performed on an Illumina HiSeq 2500 or HiSeq 2000 platform in paired-end mode. In-house bioinformatic analysis was performed. The sequences were aligned to the reference human genome (HG19/HG20). The probe size was about 2.427 MB, and the theoretical capture efficiency of the probe was 98.83%.

Data Analysis

To predict the effect of variants, we used *in silico* prediction programs to assess (PolyPhen-2, Mutation Taster, Provean, and scale-invariant feature transform [SIFT]). Pathogenic variants were under the protocol issued by American College of Medical Genetics and Genomics (ACMG) guidelines (11). The Human Gene Mutation Database (HGMD) was used to screen mutations reported in published studies. All the potential pathogenic variants observed by whole-exome and targeted panel sequencing as well as segregation analysis within family members were validated and genotyped by Sanger sequencing. Statistical analysis was performed using SPSS.25 software. All charts were completed in GraphPad Prism 8.0.2 software. Wilcoxon signed-rank test was used to explore the difference of height SDS in patients before and after rhGH treatment. The Kruskal–Wallis rank test was used to compare the height SDS and the height SDS changes after rhGH treatment in patients with collagen genes, *ACAN*, and *NPR2* mutations. $p < 0.05$ was considered statistically significant.

RESULTS

Genetic Architecture of Short Statue With Skeletal Abnormalities

Sixty-five patients were identified with genetic defects of cartilage extracellular matrix components in the 106 short-stature individuals with skeletal abnormalities, including 26 with collagen genes, 10 with *ACAN*, 4 with *COMP*, and 4 with fibrillin-1 (*FBN1*) mutation. Other frequent mutations in paracrine signaling of the growth plate development include fibroblast growth factor receptor 3 (*FGFR3*) covering 12.3% (13/106), *NPR2* covering 7.5% (8/106), and fibroblast growth factor receptor 3 (*PTH1R*) (n = 2). The remaining causal genes were associated with a fundamental cellular process, including *TRPV4* (n = 5), *SHOX* (n = 3), *KIF22* (n = 2), *TRAPPC2* (n = 1), *ARSL* (n = 1), *RUNX2* (n = 1), *CENPJ* (n = 1), *FAM111A* (n = 1), and pathogenic copy number variant (CNV) (n = 1) (**Figure 1** and **Table S1**).

Among this cohort, a total of 24 pathogenic or likely pathogenic rare variants in collagen genes were identified in 26 (24.5%) of the 106 short-stature individuals with skeletal abnormalities as per the ACMG guidelines, of which 5 variants were classified as pathogenic and 19 as likely pathogenic (**Table 1**). Type II collagenopathies were the most common. Fifteen patients, accounting for about 57.7%, had variants in the *COL2A1*, 3 had variants in the type IX collagen gene, 4 had variants in the type X collagen gene, and 2 had type XI collagen gene variants and 3 had variants in the type I collagen genes. Types of variant alleles include 18 (75.0%) missense mutations, 3 (12.5%) splicing mutations, 2 (8.2%) nonsense mutations, and 1 (4.2%) in-frame insertion mutation. Except for 4 biallelic heterozygous mutations, all the others were monoallelic heterozygous mutations. The results are summarized in **Table 1**. The mutations mainly occurred in the triple-helical region (17/24, 70.8%), followed by the C-terminal non-collagenous (NC1) domain (5/24; 20.8%) and the N-terminal non-collagenous (NC2) domain (2/24; 8.3%). Twenty-two of the variants are absent in public databases, whereas the c.1557 +5C>T variant in *COL11A2* has been identified in 1/7,442 of East Asian chromosomes by the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org), and the c.580G>A in *COL2A1* is present at an extremely low allelic frequency (4/140282) in gnomAD.

Clinical Phenotypes of Patients With Collagen Gene Mutation

The growth and phenotypic characteristics of individuals with collagen gene variations enrolled in the study are outlined in **Table 2**. The average age of patients with skeletal collagenopathies in this cohort was 7.4 ± 4.0 years for the 16 males and 10 females, and their bone age was consistent with chronological age with 6.9 ± 4.1 years, but we observed two type IX collagenopathy patients with a delayed bone age by about 3 years. The overall growth characteristics of patients with collagenopathies indicate growth retardation: four (33.3%) were born with small for gestational age (SGA); their average height Z-scores before rhGH treatment was -3.6 ± 1.4, and the calculated growth rate from 19 available individuals was 5.1 ± 1.7 cm/year. The average Z-score for

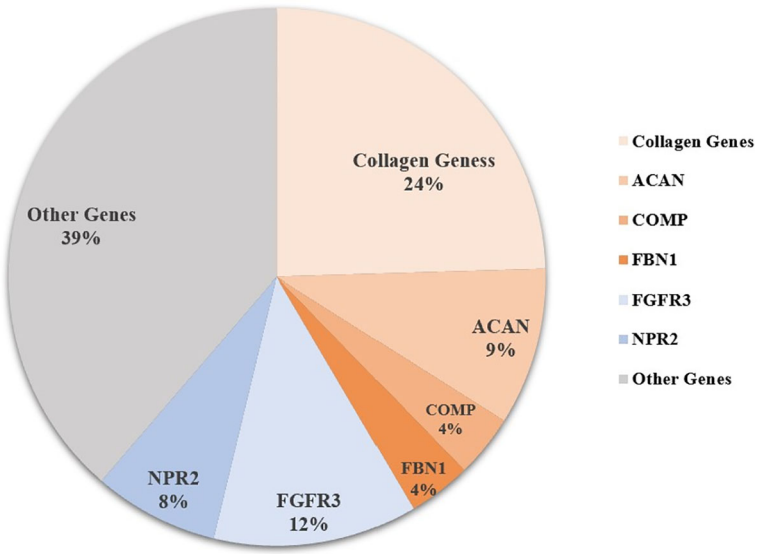


FIGURE 1 | Genetic architecture of short statue with skeletal abnormalities from a single center. Other genes include *PTH1R, TRPV4, SHOX, KIF22, TRAPPC2, ARSL, RUNX2, CENPJ, FAM111A*, CNV (copy number variant), and some unknown causative genes.

weight and body mass index (BMI) was -1.3 ± 1.2 and 0.6 ± 1.4, respectively. Thirty-nine percent (9/23) was offamilial short stature. The patients with proven collagenopathy and their affected parents had a median height Z score of -3.6 ± 1.3 and -4.1 ± 1.7, respectively.

The main clinical manifestations are growth retardation, skeletal abnormalities, and heterogeneous syndromic abnormalities involving facial, eye, hearing, and cardiac abnormalities. All patients had skeletal abnormalities, among which limb abnormalities and spinal deformities are the most common, accounting for 57.7% and 53.8%, respectively. The main manifestations of bone involvement in skeletal collagenopathies include shorting and curving of long bone or phalanges of extremities, metaphyseal dysplasia of spine and limbs, arthrogryposis, joint laxity, scoliosis, or kyphosis. In addition, chest deformity, mainly presented as pectus carinatum, was also observed in 8 patients (30.8%). Fifty-eight percent of cases had facial abnormalities commonly observed in patients, mainly a low nasal bridge, high-arched palate, small jaw, or big or prominent ears. Cleft palate was observed in two patients with type II and one with type XI collagen gene mutations, and blue sclera was observed in two patients with type I and one with type IX collagen gene mutations. In addition, there were a small number of patients with other system defects, such as three with heart defects, three with congenital cataracts, strabismus, or amblyopia, and two with mixed deafness or ear deformity and congenital aural atresia.

Phenotypic and Genotypic Analyses of Short Statue With Skeletal Abnormalities

Our data obtained from 15 probands with 12 kinds of *COL2A1* mutations showed that the phenotypic spectrum of *COL2A1* mutations included spinal deformity, abnormal cartilage development, midface hypoplasia, and ocular abnormalities. Almost all showed spine deformity including scoliosis, lordosis, dysplasia, and osteoporosis. The clinical manifestations of three boys (P.11, P.12, P.13 shown in **Table S2**) from three independent families with the same hot spot mutation (p.Arg989Cys) were consistent with spondyloepimetaphyseal dysplasia Strudwick type (SEDC) (MIM #184250), of which P.12 and P.13 had more severe skeletal manifestations and were diagnosed at 5.3 and 6.8 years of age, respectively, while P.11 was the milder type and diagnosed at a later age of 14.8 years. One novel missense variant (p.Ala194Thr) was identified and confirmed to segregate with the autosomal dominant short-stature phenotype in a family (P.3), which showed only short stature and flat round face with no obvious skeletal deformity in P.4. Besides, P.7 carries pathogenic variants in both *COL2A1* and *COL9A2* mutations resulting in a more severe skeletal deformity and short-trunk dwarfism, while the other two patients (P.16, P.17) in our study only carrying *COL9A1/COL9A2* mutation had very mild symptoms, with only mild chondrodysplasia.

In the data obtained from three probands in two independent families with *COL10A1* mutations, one missense variant 1766T>G (p.Phe589Cys) was *de novo* paternity and maternity confirmed in two identical twin brothers (P.18, P.19), and one truncating mutation c.1858_1865del CCTGTAAT (p.Pro620Valfs*4) was confirmed to segregate with the autosomal-dominant short-stature phenotype in five affected family members (P.21). Both types of mutations were located in the NC1 domain. All three probands and affected relatives exhibited typical metaphyseal chondrodysplasia type Schmid (SMCD) (MIN #156500) phenotypes with short bowed limbs, valgus knees, pronounced lumbar lordosis, posterior flexion of hips, enlarged large joints, and a faltering gait, which were consistent with radiographical findings.

TABLE 1 | In silico analysis and ACMG/AMP classification of collagen gene identified variants.

cDNA variant	Mutation status	Domain	Mutation taster	Polyphen-2	Provean	SIFT	ACMG-AMP classification
COL2A1							
c.196G>A(Asp66Asn)	Comhete.	NC2 domain	0.999/D	0.094/B	-0.83/N	0.17/N	Likely pathogenic
c.580G>A(p.Ala194Thr)	Heter.	NC2 domain	0.897/D	0.897/PD	-0.94/N	0.06/T	Likely pathogenic
c.1124G>T(p.Gly375Val)	Heter.	Triple-helical region	0.999/D	0.999/PD	-6.86/D	0.00/D	Likely pathogenic
c.1160G>A(p.Gly387Asp)	Heter.	Triple-helical region	0.999/D	0.999/PD	-5.33/D	0.00/D	Likely pathogenic
c.1202C>T (p.Pro401Leu)	Heter.	Triple-helical region	0.999/D	0.999/PD	-1.24/N	0.07/T	Likely pathogenic
c.1680+8_1680+9delGCinsTA	Heter.	Triple-helical region	NA	NA	NA	NA	Likely pathogenic
c.1789G>A(p.Gly597Arg)	Heter.	Triple-helical region	0.999/D	1.000/PD	-6.93/D	0.00/D	Likely pathogenic
c.2302-10C>T	Comhete.	Triple-helical region	NA	NA	NA	NA	Likely pathogenic
c.2401G>A(p.Gly801Ser)	Heter.	Triple-helical region	0.999/D	0.999/PD	-5.46/D	0.00/D	Likely pathogenic
c.2725G>A(p.Gly909Ser)	Heter.	Triple-helical region	0.999/D	0.999/PD	-5.07/D	0.00/D	Likely pathogenic
c.2965C>T(p.Arg989Cys)	Heter.	Triple-helical region	0.999/D	0.998	-7.09/D	0.00/D	Pathogenic
c.3472G>T(p.Gly1158Cys)	Heter.	Triple-helical region	0.999/D	1.000/PD	-8.41/D	0.00/D	Likely pathogenic
COL9A1							
c.2636C>A(p.Pro879His)	Heter.	Triple-helical region	0.999/D	0.997	-3.20/D	0.01/D	Likely pathogenic
COL9A2							
c.185C>T(p.Pro62Leu)	Heter.	Triple-helical region	0.999/D	0.05/B	-3.46/D	0.076/T	Likely pathogenic
c.1243G>C(p.Gly415Arg)	Heter.	Triple-helical region		1.000/PD	-6.67/D	0.00/D	Likely pathogenic
COL10A1							
c.1471C>T(p.Pro491Ser)	Heter.	Triple-helical region	0.999/D	0.509/D	-1.86/N	0.09/T	Likely pathogenic
c.1766T>G(p.Phe589Cys)	Heter.	NC1 domain	0.999/D	1.000/PD	-4.00/D	0.01/D	Likely pathogenic
c.1858_1865del CCTGTAAT (p.Pro620Valfs* 4)	Heter.	NC1 domain	1.000/D	NA	NA	NA	Pathogenic
COL11A1							
c.739G>T(p.Ala247Ser)	Heter.	NC1 domain	0.999/B	1.000/B	-0.44/N	0.80/T	Likely pathogenic
COL11A2							
c.1557+5C>T	Heter.	Triple-helical region	NA	NA	NA	NA	Likely pathogenic
COL1A1							
c.1386delT (p.Ala463Leufs*78)	Heter.	Triple-helical region	1.000/D	NA	NA	NA	Pathogenic
COL1A2							
c.2121_2122ins GCTGGTCCT (Pro707_Arg708insAlaGlyPro)	Comhete.	Triple-helical region	1.000/D	NA	NA	NA	Pathogenic
c.3583T>C(p.Cys1195Arg)	Comhete.	NC1 domain	0.999/D	1.000/PD	-10.31/D	0.00/D	Likely pathogenic
c.3997A>G(p.Thr1333Ala)	Heter.	NC1 domain	0.999/D	0.999/PD	-4.07/D	0.00/D	Likely pathogenic

Comhete., compound heterozygous; Heter, heterozygous; NC1, C-terminal non-collagenous; NC2, N-terminal non-collagenous; D, deleterious; B, benign; N, neutral; PD, possibly/probably damaging; T, tolerance; NA, not available.

In addition, the proband with truncating mutations was born with flexion of the legs and had more severe forms of SMCD with additional manifestations such as short neck, pectus carinatum, beaded ribs, and widened epiphysis of the ribs. The two cases caused by missense variants exhibited relatively late-onset ages at around 2 years of age and mild or moderate manifestations.

Two novel variants in the type XI collagen gene were identified with one missense variant of *COL11A1* [c.739G>T (p.Ala247Ser)] and one splice site alteration of *COL11A2* (c.1557 +5C>T). The girl (P.22) with the *COL11A1* mutation had a phenotype consistent with mild Marshall syndrome (MIM #154780), with midfacial hypoplasia, cleft palate, a less severe ocular presentation, but striking ocular hypertelorism, and short stature with spondyloepiphyseal dysplasia (12). The boy (P.23) with a *COL11A2* splice site alteration was characterized by Stickler syndrome (MIM #108300) with congenital cataract, sensorineural deafness, relatively short extremities with elbows valgus and joints pain, and typical midface hypoplasia.

TABLE 2 | Baseline characteristics of individuals with skeletal collagenopathies.

	Type II collagen	Type IX collagen	Type X collagen	Type XI collagen	Type I collagen	Total
Demographic characteristics						
Male/female, n	9/6	2/0	3/1	1/1	1/2	16/10
Median age (n = 26)	7.6 ± 4.5	11.7 ± 2.2	7.5 ± 2.7	4.7 ± 0.1	5.8 ± 2.5	7.4 ± 3.9
Bone age (n)	7.3 ± 4.4 (11)	8.8 ± 2.8 (2)	6.7 ± 1.9 (4)	NA	5.7 ± 3.3 (3)	6.9 ± 4.1 (20)
Growth characteristics						
Growth velocity (n)	5.0 ± 1.5 (11)	5.8 ± 0.8 (2)	4.9 ± 2.5 (2)	4.8 ± 1.2 (2)	5.8 ± 2.3 (2)	5.1 ± 1.7 (19)
Height Z-scores (n = 26)	-4.1 ± 1.6	-3.5 ± 0.9	-2.7 ± 0.4	-3.2 ± 0.2	-2.6 ± 0.4	-3.6 ± 1.4
Weight Z-scores (n = 26)	-1.2 ± 1.4	-1.7 ± 50.7	-0.5 ± 0.8	-2.4 ± 0.7	-1.7 ± 0.7	-1.3 ± 1.2
BMI Z-scores (n = 26)	1.0 ± 1.2	-0.7 ± 0.8	1.6 ± 0.6	-0.8 ± 1.0	-0.8 ± 0.0	0.6 ± 1.4
IGF-1 Z-scores (n)	-0.43 ± 1.3 (10)	-5.1 ± 1.8 (2)	0.25 ± 1.8 (3)	-2.6 ± 1.1 (2)	-0.3 ± 1.2 (3)	-1.0 ± 2.1 (20)
SGA (n)	1 (11)	0 (1)	1 (2)	1 (1)	1 (3)	4 (18)
Family history (n)	6 (12)	0 (2)	2 (4)	0 (2)	1 (3)	9 (23)
Syndromic defects (n = 26)						
Midface hypoplasia	8	2	0	2	3	15
Thoracic deformity	6	0	1	1	0	8
Limb abnormalities	9	0	3	0	3	15
Scoliosis	8	1	4	0	1	14
Joint hypermobility	1	0	1	0	2	4
Congenital heart defect	0	1	0	0	2	3
Ocular abnormalities	1	0	0	1	1	3
Hearing loss	0	1	0	1	0	2
Cleft palate	2	0	0	1	0	3

BMI, body mass index; IGF-1, insulin-like growth factor I; SGA, small for gestational age; NA, not avaliable.

Three patients with mutations in genes encoding type I collagen (*COL1A1* and *COL1A2*) were identified, and their skeletal abnormalities were characterized primarily by osteoporosis, with less common abnormalities in the limb bones and skull. In addition to skeletal abnormalities, they also have abnormalities in many organs, such as cardiovascular, joints, ligaments, midface development, and ocular anomalies. One patient (P.26) with biallelic heterozygous mutations [c.2121_2122insGCTGGTCCT (p.Pro707_Arg708ins AlaGlyPro) and c.3583T>C (p. Cys1195Arg)] and one (P.24) with heterozygous truncating mutation [c.1386delT (p. Ala463Leufs*78)] had more severe osteogenesis imperfecta, such as early-onset motor retardation, heart defects, reduced thoracolumbar bone density, and obvious joint relaxation, than did the heterozygous missense mutations [c.3997A>G (p. Thr1333Ala)] (P.25).

The clinical phenotypes of other skeletal abnormality-related short stature were also briefly summarized based on our recent report (13–15). Heterozygous mutations in *ACAN* can lead to spondyloepiphyseal dysplasia, Kimberley type (MIM #608361), or osteochondritis dissecans (MIM #165800), which was consistent with the clinical findings of the 10 patients in our cohort, presenting as mild midface hypoplasia, short neck, thoracic deformity, spine malformation, short fingers/toes, short metacarpal bones, internal rotation of the elbow (contrast to cubitus valgus), and café-au-lait spots, and none of them complained of bone or joint pain. Four patients with *COMP* mutations exhibited typical pseudoachondroplasia (PSACH) (MIM #177170) with severe short-limb dwarfism, joint pain and stiffness, and early-onset osteoarthritis, and 4 patients with mutations in *FBN1* represented as acromelic dysplasia (MIM # 102370) shared severe short stature, short hands and feet, and joint limitations. Biallelic variations of *NPR2* mutation can cause acromesomelic dysplasia, Maroteaux type (AMDM) (MIM #602875), while monoallelic variants result in short stature with non-specific skeletal deformities and Miura-type osteochondral dysplasia. Autosomal dominant mutations in *FGFR3* causing achondroplasia (ACH) (MIM #100800) were found in 13 of our cohort. They appeared as short stature resulting from the shortening of the limbs with proximal segments affected disproportionally. In addition, the typical broad or protruding forehead, lumbar lordosis, and sacral kyphosis were seen in all these patients. Among the 8 cases of *NPR2* mutation in our study, except for one case with compound heterozygous mutation characterized by disproportionate short stature, mesomelic limb shortening, and shortened and broadened fingers and toes, conforming to AMDM, the other 7 cases were heterozygous mutation with or without disproportionate short stature, facial anomalies, and non-specific skeletal deformities, including mesomelic limb shortening, cubitus valgus, brachydactyly, shortened metacarpals or metatarsals, clinodactyly, and cone-shaped epiphysis.

Comparison of Collagen Gene-Related Short Stature With Other Short-Stature Genes and Growth Response to rhGH Treatment

There were 9 patients with collagen gene mutation who had received rhGH treatment, two of whom had combined treatment with gonadotropin-releasing hormone agonists (GnRHa) (**Table 3**). The initial age of treatment was 7.9 ± 3.5 years. After 1 year of treatment, the growth rate increased from 6.0 ± 1.6 to 9.0 ± 1.3 cm/years, and the average height Z score significantly increased from -3.2 ± 0.9 to -2.5 ± 1.0 (p <.01). Their average height Z score at the last follow-up was significantly increased to -2.2 ± 1.2 (p <.001) (**Figure 2**). The average duration of treatment was 2.9 ± 1.9 years, and three of them were treated discontinuously. Two cases (P.5, P.10) of scoliosis occurred after initial treatment, both of which discontinued the therapy for

TABLE 3 | Overview of patients with skeletal collagenopathies with rhGH treatment.

Patient ID	P.2	P.15	P.11	P.5	P.10	P.17	P.20	P.23	P.24
Mutation	*COL2A1*	*COL2A1*	*COL2A1*	*COL2A1*	*COL2A1*	*COL9A2*	*COL10A1*	*COL11A2*	*COL1A2*
Sex	F	F	F	M	M	M	M	M	M
Age of treatment (y)	11.25	11	3.75	13.67	4	10.08	5.67	4.58	7.33
Duration (year)	1.7[a]	1.25	6.00	0.2[b] + 0.92[c]	1.17[b] + 2[c]	2.83	1.25 + 1[a,c]	6.50	1.5
Growth velocity before (cm/year)	3.5	4.4	8	4.5	NA[d]	6.5	7.4	6	8
Height SDS before	-2.44	-2.65	-4.03	-4.51	-4.52	-2.61	-2.00	-3.35	-2.88
Growth velocity after 1 year	9.00	9.40	9	NA[d]	6.94	8.6	11.7	9.34	8.2
Height SDS at 1 year	-1.90	-2.17	-2.8	-4.25	-3.98	-1.93	-1.20	-2.55	-2.00
Height SDS change at 1 year	0.54	0.48	1.20	0.31	0.54	0.68	0.8	0.80	0.88
Last available height SDS	-1.47	-1.47	-1.77	-4.28	-4.7	-1.86	-1.19	-1.00	-1.79
Total height SDS change	0.97	0.97	2.26	0.23	-0.18	0.75	0.2	1.71	1.09

[a]*Plus triptorelin.*
[b]*Accompanied by scoliosis during treatment.*
[c]*Discontinuous treatment.*
[d]*NA, not available.*

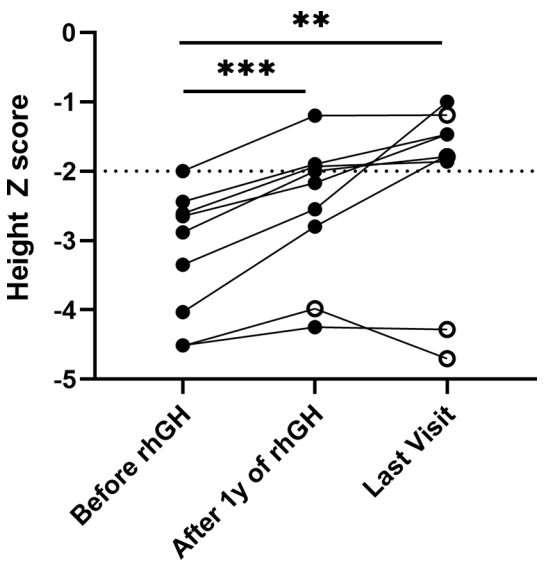


FIGURE 2 | Height Z score in patients with collagenopathies with rhGH treatment. The median follow-up time was 2.25 years with a range of 1.12 to 6.50 years. Solid black dots indicate continued treatment up to the last follow-up, and hollow black dots indicate discontinuous treatment, **p <.01, ***p <.001.

orthopedic evaluation (P.10 underwent spinal orthopedic treatment) and continued treatment. One girl (P.20) received rhGH at age of 5.7 and discontinued for personal reasons 1.3 years later, followed by the diagnosis of central precocious puberty with advanced bone age (+2~3 years), breast development at 7 years of age, and menophania at 9.8 years of age, and rhGH treatment was restarted combined with GnRHa for 1 year at 10 years of age, with poor therapeutic response. After exclusion of these three patients from growth response, the six remaining patients had an average improvement in a height Z score of 1.3 ± 0.5 (range, 0.8 to 2.3). The height of the two patients who had been treated for more than 6 years significantly increased their height Z score by 2.26 and 1.71, respectively. No abnormalities or side effects were observed throughout the treatment.

We pooled demographic and treatment information of all short-stature patients with skeletal abnormalities both in our cohort and partly from the literature review, including other extracellular matrix component genes (*ACAN*, *COMP*, and *FBN1*) and paracrine signaling genes (*FGFR3* and *NPR2*), in which a subset of the 29 patients with *ACAN* mutation and 21 patients with *NPR2* mutation were from the literature review, based on previous reports (**Table 4**) (13, 15). A total of 121 affected individuals diagnosed genetically are shown in **Table 4**. The gender proportion of males to females was 1.7 (73 males and 46 females). Their height Z score was −3.8 ± 2.0 from 114 affected individuals. The height Z score of males (70 cases) versus females (44 cases) was -3.9 ± 0.2 versus -3.7 ± 0.3 (p = .747), indicating that there is no difference in height between males and females. Six of the patients were adults with a height Z score of -6.8 ± 1.2, which was significantly lower than the 100 juvenile individuals with a height Z score of -3.7 ± 0.2 (p = .001),

TABLE 4 | Comparison of collagen gene-related short stature with other short stature genetic architecture.

		n	Age (year)	Sex		Height Z score		n	Treatment		Height Z score change	p
				Male	Female				Before	After		
Extracellular matrix	Collagen Genes	26	6.63 [3.67–10.25]	16	10	-3.62 ± 1.40	Collagen Genes	9	-3.22 ± 0.93	-2.53 ± 1.00	0.69 ± 0.28	**<0.001**
	ACAN [a]	29	9.71 [5.53–12.2]	20	9	-2.85 ± 1.01[d]	*ACAN*	29	-2.85 ± 1.00	-2.22 ± 1.12	0.63 ± 0.71	**<0.001**
	COMP [b]	27	5.60 [3.4–15.0]	15	10	-5.41 ± 2.71[d]	–					
	FBN1	4	5.83 [4.00–11.37]	2	2	-4.99 ± 0.98	–					
Paracrine signaling	*FGFR3*	13	5.83 [3.09–9.75]	5	8	-4.37 ± 1.80	*FGFR3*	4	-4.01 ± 2.27	-3.15 ± 1.47	0.86 ± 0.97	0.547
	NPR2 [c]	21	7.00 [4.83–10.50]	14	7	-3.12 ± 0.79	*NPR2*	21	-3.12 ± 0.79	-1.98 ± 1.04	1.14 ± 0.68[e]	**<0.001**
Total		121	6.75 [4.00–11.17]	73	46	-3.75 ± 1.96	Total	63	-3.07 ± 1.05	-2.24 ± 1.11	0.82 ± 0.70	**<0.001**
						<0.001	***p***		0.438	0.427	**0.014**	

Data are expressed as median [interquartile range] and mean ± standard deviation (number of patients for whom the data were available).
Footnotes a–e indicate statistics within each group.
[a,b]Data were obtained from Liang et al. (13, 14).
[c]Data were obtained from Ke et al. (15).
[d]Significant (p <0.05 or less) vs. collagen genes.
[e]Significant (p <0.05 or less) vs. ACAN.
ACAN, aggrecan; COMP, cartilage oligomeric matrix protein; FBN1, fibrillin 1; FGFR3, fibroblast growth factor receptor 3; NPR2, natriuretic peptide receptor 2; SDS, standard deviation score.
The bold values mean p <0.05 or less.

suggesting that the height impairment worsened with age and would be more severely affected in adults without any treatment. In addition, when the affected individuals were divided into extracellular matrix maintenance and paracrine signaling of the growth plate and long bone development according to physiological etiologies of short stature, there was no significant difference in their height impairment (p = .683). We compared the height Z score of collagenopathy patients with other different-causing gene mutations and found that the *ACAN* mutation resulted in milder short stature than the collagen gene mutation (p = .021), while the *COMP* mutation was the most severe (p = .009) (**Table 4** and **Figure 3**).

In short-stature patients with *ACAN* and *NPR2* mutation, rhGH had a significant effect on height gain as reported previously (**Figure 4**) (13, 15). In *ACAN*-related short stature, rhGH treatment significantly increased height and the height Z score (from -2.9 ± 1.0 to -2.2 ± 1.1) after 2.8 ± 0.4 years of administration. For NPR2-related short stature, height Z scores were significantly improved from -3.1 ± 0.8 to -2.0 ± 1.0 after 3.8 ± 0.6 years of treatment. Moreover, the growth response for rhGH treatment in *ACAN*-related short stature was better than *NPR2* (p = .014) (**Figure 4**). For *FGFR3*-related short, height Z scores were improved from -4.0 ± 2.3 to -3.2 ± 1.5, but this was not significant. However, given the severe PSACH phenotype caused by *COMP* mutations, limited benefit, and possibly serious complications, growth-promoting therapies were not recommended.

DISCUSSION

In this study, we performed NGS in short-stature patients with skeletal abnormalities and identified causal variants of skeletal collagen genes in 26 (24.5%) of the 106 individuals, among which 15 patients (14%) carried *COL2A1* mutations. Our molecular diagnosis rate of patients with skeletal abnormalities was 76.4% (81/106) and skeletal collagen genes accounted for 24.5% (26/106). *COL2A1* mutations were detected in 15 patients (14%), which was slightly higher than that of a recent NGS study on 82 short Chinese patients with clear signs of bone dysplasia (positive rate = 11%) (16). A recent study evaluating oligosymptomatic

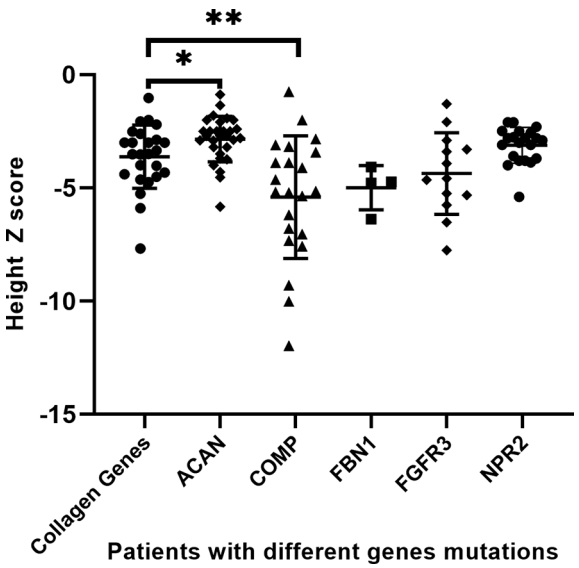


FIGURE 3 | Comparison of different gene mutations leading to growth retardation. *p <.05, **p <.01.

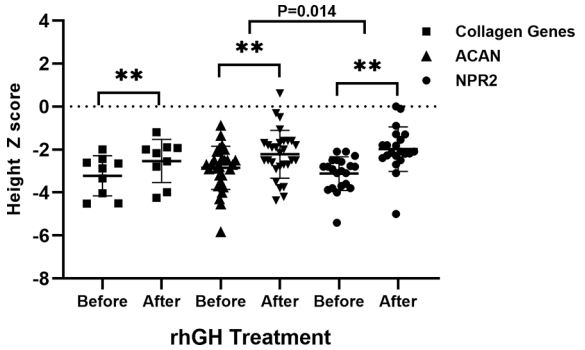


FIGURE 4 | Comparison of growth response to rhGH between patients with collagen gene, *ACAN*, and *NPR2* mutations. **p <.01.

collagenopathies yielded a lower molecular diagnostic rate of 11.5% in 87 FSS patients treated with rhGH (17). Meanwhile, our study revealed the genetic architecture of short stature with skeletal abnormalities and proposed that mutations of collagen genes (especially *COL2A1*), *FGFR3*, *ACAN*, *NPR2*, *COMP*, and *FBN1* are common for short stature due to skeletal abnormalities in outpatient clinics in pediatric endocrinology. More importantly, we provided initial information about the phenotypical spectrum of collagenopathies by proving that short stature with skeletal abnormalities and heterogeneous syndromic abnormalities may be caused by mutations in the collagen genes.

Longitudinal growth of the skeleton is a result of endochondral ossification taking place in the epiphyseal growth plates of the long bones. The cartilaginous growth plate consists of extracellular matrix (ECM) and linear columns of differentiated chondrocytes that are organized into resting, proliferating, mature, and hypertrophic zones, which are continuously replaced by trabecular bone with the increase in length (1, 18, 19). Thus, multiple processes involved in the growth plate and long bone development, including basic cellular processes, extracellular matrix maintenance, paracrine signaling, and hormonal signaling, may underlie the distinct genetic architectures and physiological etiology of short stature with skeletal abnormalities. Collagens are a family of structurally related proteins that play a wide variety of roles in the ECM, are characterized by a basic structural coiled-coil right-handed triple helix, and are composed of three polypeptide chains (α chains). Both type II and X collagens are homotrimers composed of 3 identical chains encoded by the *COL2A1* and *COL10A1* genes, respectively. Both type IX and XI collagens are heterotrimers encoded by 3 different chains of *COL9A1*, *COL9A2*, *COL9A3* genes, and *COL11A1*, *COL11A2*, and *COL11A3*, respectively. Type I collagen is also a heterotrimeric molecule encoded by *COL1A1* and *COL1A2*. These genes encode procollagens, which are synthesized in the endoplasmic reticulum and contain a short N-telopeptide non-triple-helical (NC2) domain, a long triple-helical domain, and a short C-telopeptide non-triple-helical (NC1) domain, and then posttranslational modification generates mature collagens, which are secreted into the extracellular matrix (20, 21). In our study, of the 23 variants, 16 occurred in the triple-helical domain, highlighting the importance of this domain in the collagen genes. Consistent with previously reported results, all pathogenic variants of *COL10A1* in our study were in the NC1 domain, which contains motifs required for normal assembly of the collagen trimer (22). In addition, type II collagen fibrils have covalently linked type IX fibrils on their surface and at their core is a fibril template of type XI collagen. This association of types II, IX, and XI collagens can explain some of the phenotypic overlaps among the resulting conditions (23).

Since collagen is one of the components of many tissues and organs, genetic defects of collagen formation can affect almost every organ system and tissue in the body, and the clinical features often overlap, showing a variable syndrome in addition to bone phenotype. Patients with skeletal collagenopathies described in our study typically manifest growth retardation, skeletal abnormalities, and heterogeneous syndromic abnormalities involving facial, eye, hearing, and cardiac abnormalities, including osteogenesis imperfecta, a variety of chondrodysplasias, rarely, some forms of osteoporosis, osteoarthritis, joint hypermobility, and extra-skeletal features, for example, myopia, astigmatism, cataracts, sensorineural and conductive hearing loss, and mitral or tricuspid regurgitation. Other frequent gene mutations related to skeletal development, including *FGFR3*, *ACAN*, *NPR2*, *COMP*, and, *FBN1*, usually cause varying degrees of short stature, with or without other mild abnormalities, such as slight growth ratio imbalance and skeletal non-specific abnormalities (such as short finger/toe, short thumb, or midfacial dysplasia) (7, 8). In addition, one patient (P.6) with *COL2A1* mutation showed unexpected obesity and severe skeletal deformities and arthrogryposis, suggesting that weight gain and obesity may also be major concerns of epiphyseal dysplasia and contribute to the morbidity associated with joint problems. The underlying collagen mutation disrupts normal cartilage architecture, resulting in premature cartilage degeneration, and patients with these disorders often require joint replacement in the third to fourth decades of life (21). Both of our two patients with type IX collagen gene mutation showed a mild type of multiple epiphyseal dysplasias, which may be related to its high clinical heterogeneity and complex genetic background, and the late onset of the phenotype may also be one of the reasons as there was also a case that reported that symptoms did not appear until the age of 45 (24). Type X collagen is synthesized exclusively by hypertrophic chondrocytes in the cartilage growth plates of growing bones undergoing endochondral ossification, and its role of an extracellular scaffold or in the mineralization of hypertrophic growth plate has been proposed. Pathogenic variants in *COL10A1* cause reduced levels of functional type X collagen in the growth plate and contribute to the development of SMCD phenotypes, a disorder characterized by dwarfism and an expanded growth plate hypertrophic zone, which was seen in three of our patients with two novel missense and truncating variants (21). In the

2 patients with type XI collagen gene mutation, besides the typical skeletal and orofacial manifestations, P.22 with *COL11A1* missense mutation showed a less severe ocular presentation, while the boy with a *COL11A2* splice site alteration presented with obvious ocular anomalies of congenital cataracts. These findings are different from previous variants associated with Stickler syndrome caused by mutations in genes encoding type XI collagens, in which ocular anomalies are predominantly present in *COL11A1* mutation and the *COL11A2* heterozygous mutation usually causes non-ocular Stickler syndrome (25).

Although precise genotype–phenotypic correlations in collagen genes have not yet been established, we have made some interesting findings. Phenotypic severity might vary among patients with the same mutation, and in patients with *COL2A1* mutations, age at diagnosis might also be associated with disease severity, which was consistent with a previous study of *COL2A1* based on a large database (20). The genotype–phenotype correlation of *COL10A1* cases in this study was also consistent with previous reports (26, 27). Most of the identified mutations were present in the NC1 domain, which had motifs that control the formation of stable collagen X molecules by promoting the formation of the triple helix. The three cases of *COL10A1* mutation we reported all showed SMCD with short-limbed short stature, bowed legs, and a waddling gait, while two cases caused by missense variants exhibited relatively late-onset ages and moderate manifestations than the truncating one. More importantly, we compared the demographic and growth characteristics of patients with different disease-causing genes and found that children with skeletal dysplasia were usually severely short, and the heights of adults who did not receive treatment were significantly more impaired. In addition, among the short-stature individuals with causing genes, compared with individuals with collagen gene mutations, those with *ACAN* mutations showed a milder short stature, while PSACH with *COMP* mutations was at the severe end of the dwarfism spectrum and was associated with significant limb shortening (28). Overall, we still suggest that a larger phenotypic spectrum of collagen gene mutations would help construct a solid basis for further research of the genotype–phenotype correlation.

GH therapy has been introduced in several syndromic disorders with short stature, i.e., Noonan syndrome, Prader–Willi syndrome (PWS), and Silver–Russell syndrome (SRS), while there are limited data on the effect of rhGH treatment on children with short stature and skeletal dysplasia (29–31). Recently, we demonstrated a good effect of rhGH treatment in patients with *NPR2* heterozygous mutation, and the efficacy was negatively correlated with the initial age of treatment and was associated with gender and the gene positions of mutation (15). A study evaluating the efficiency of rhGH treatment with collagenopathy in a cohort also demonstrated a height Z score improvement from a median of –3.1 to –2.6 and to –2.2 after 1 and 3 years of therapy, respectively (17). Our study provides new evidence for the evaluation of rhGH therapy for skeletal collagenopathies. Consistent with previous results, the individual height Z score benefit after rhGH replacement varied considerably in this study (range: -0.18 to 2.26). During an average duration of 2.8 ± 2.1 years, the height Z score of 9 patients who received rhGH treatment improved from a median of -3.2 ± 0.9 to -2.2 ± 1.3 at the last follow-up, respectively. The most significant height Z score improvement of 2.3 and 1.7 was also seen in two patients who had been treated for more than 6 years. Limb and spinal deformities were a problem frequently seen in children with skeletal collagenopathies (57.7% and 53.8%). There was a concern about whether rhGH treatment would increase the frequency or severity of this finding, especially scoliosis. Two cases (P.5, P.10) of scoliosis occurred after initial treatment, both of which discontinued the therapy for orthopedic evaluation (P.10 underwent spinal orthopedic treatment) and continued treatment. This was also the main reason why rhGH is not recommended for patients with *COMP* mutations leading to PSACH, usually accompanied by severe osteoarthropathy. A previous clinical trial also demonstrated that in cases of PSACH, the height Z score was worse after rhGH therapy (32). Except for those two patients with the *COL2A1* mutation who developed scoliosis after initiating treatment, no exacerbation of scoliosis or other skeletal deformities was observed in the remaining patients. However, we do not think that the occurrence of this scoliosis was completely caused by rhGH treatment, because both patients carry hotspot mutation of *COL2A1* leading to SEDC, and the incidence of this disease is relatively high, which has been reported as 48% of 93 patients with molecularly confirmed SEDC or a related disorder in the previous literature (33). In addition, for the general short-stature population with skeletal dysplasia, *ACAN*-related short stature was more responsive to rhGH treatment than *NPR2*-related short stature, and significant height improvement was not seen in FGFR3-related short-stature patients in this cohort. While the effectiveness of rhGH for ACH caused by *FGFR3* mutation had been proposed in previous cohort studies (34), the very small sample size in our study may be the reason for the difference. A similar difference in rhGH efficacy was also observed in two patients with osteogenesis imperfecta patients who received bisphosphonates with or without rhGH. During the 2-year follow-up, the height Z score of P.24 combined with rhGH therapy was improved from -3.0 to -1.8, while the height Z score of patients treated with bisphosphonates alone was decreased from -2.0 to -3.3, which reveals the effectiveness of bisphosphonate combined with rhGH in patients with osteogenesis imperfecta. Moreover, there are quite a few studies that have confirmed the effectiveness of the combination therapy, not only in terms of growth velocity but also in bone mineral density and bone turnover (35–37). There were no complications such as fractures in both patients. Overall, this evidence suggests that rhGH treatment tolerability and efficacy in improving growth in patients with skeletal abnormalities vary greatly and careful consideration of indications for therapy and cautious observation during therapy are crucial for each patient.

Potential limitations of this work warrant consideration. First of all, in terms of molecular genetic testing approaches, WES

may not detect large CNVs. Therefore, multiple ligation-dependent probe amplification (MLPA) or chromosomal microarray (CMA) analysis should be performed to screen for large deletions and insertions in genes belonging to the short-stature gene panel. In addition, some of the newly discovered variants still lack further validation, so we only evaluated their pathogenicity according to the ACMG guidelines, combined with their clinical phenotypes, pedigree verification, and *in silico* prediction programs. Furthermore, there are signs or clinical manifestations that have not been identified in some of our patients due to young age or too short follow-up. It is also worth mentioning that, unfortunately, we did not routinely assess bone mass in some children without a clear history of fractures. Moreover, some evidence for segregation of the variants with short stature within the families is lacking in some suspect pedigrees because of some unavailable relatives, which can strongly support the mutations' pathogenicity. Lastly, more accurate genotype–phenotype correlations and evidence for the treatment of recombinant growth hormone deserve further study in a larger cohort of skeletal collagenopathies children with short stature.

In conclusion, skeletal collagenopathies are relatively frequent in syndromic-related short stature, and screening for collagen mutations should be considered in short-stature children with skeletal abnormalities. Although long-term studies evaluating rhGH treatment are insufficient and large cohort studies regarding rhGH dose, the optimal age to start treatment, and adverse events are lacking, initial information provided by our study about the efficacy of rhGH treatment for skeletal collagenopathies indicates an improved growth rate and height. Before starting rhGH treatment, patients with collagenopathy-related short stature should be extensively evaluated, and close monitoring of adverse reactions such as scoliosis is required.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: National Genomics Data Center (NGDC), PRJCA008063.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Peking Union Medical College Hospital Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MC and HZ designed the study. MC, HM, and HL collected the data. MC and XK conducted the data collection and analysis. FG guided the experimental study. MC and HZ drafted the manuscript. HY, LW, LD, SC, and HP interpreted the data and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the CAMS Innovation Fund for Medical Sciences (CIFMS 2021-I2M-1-003).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.820001/full#supplementary-material

REFERENCES

1. Krakow D, Rimoin DL. The Skeletal Dysplasias. *Genet Med* (2010) 12(6):327–41. doi: 10.1097/GIM.0b013e3181daae9b
2. Baron J, Sävendahl L, De Luca F, Dauber A, Phillip M, Wit JM, et al. Short and Tall Stature: A New Paradigm Emerges. *Nat Rev Endocrinol* (2015) 11 (12):735–46. doi: 10.1038/nrendo.2015.165
3. Grunauer M, Jorge AAL. Genetic Short Stature. *Growth Horm IGF Res* (2018) 38:29–33. doi: 10.1016/j.ghir.2017.12.003
4. Kruszka P, Porras AR, Addissie YA, Moresco A, Medrano S, Mok GTK, et al. Noonan Syndrome in Diverse Populations. *Am J Med Genet A* (2017) 173 (9):2323–34. doi: 10.1002/ajmg.a.38362
5. Laron Z, Werner H. Laron Syndrome - A Historical Perspective. *Rev Endocr Metab Disord* (2021) 22(1):31–41. doi: 10.1007/s11154-020-09595-0
6. Jee YH, Baron J, Nilsson O. New Developments in the Genetic Diagnosis of Short Stature. *Curr Opin Pediatr* (2018) 30(4):541–7. doi: 10.1097/mop.0000000000000653
7. Plachy L, Strakova V, Elblova L, Obermannova B, Kolouskova S, Snajderova M, et al. High Prevalence of Growth Plate Gene Variants in Children With Familial Short Stature Treated With Gh. *J Clin Endocrinol Metab* (2019) 104 (10):4273–81. doi: 10.1210/jc.2018-02288
8. Gkourogianni A, Andrew M, Tyzinski L, Crocker M, Douglas J, Dunbar N, et al. Clinical Characterization of Patients With Autosomal Dominant Short Stature Due to Aggrecan Mutations. *J Clin Endocrinol Metab* (2017) 102 (2):460–9. doi: 10.1210/jc.2016-3313
9. Jobling R, D'Souza R, Baker N, Lara-Corrales I, Mendoza-Londono R, Dupuis L, et al. The Collagenopathies: Review of Clinical Phenotypes and Molecular Correlations. *Curr Rheumatol Rep* (2014) 16(1):394. doi: 10.1007/s11926-013-0394-3
10. Li H, Durbin R. Fast and Accurate Short Read Alignment With Burrows-Wheeler Transform. *Bioinformatics* (2009) 25(14):1754–60. doi: 10.1093/bioinformatics/btp324
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* (2015) 17(5):405–24. doi: 10.1038/gim.2015.30
12. Shanske AL, Bogdanow A, Shprintzen RJ, Marion RW. The Marshall Syndrome: Report of a New Family and Review of the Literature. *Am J Med Genet* (1997) 70(1):52–7. doi: 10.1002/(sici)1096-8628(19970502)70:1<52::aid-ajmg11>3.0.co;2-w
13. Liang H, Miao H, Pan H, Yang H, Gong F, Duan L, et al. Growth-Promoting Therapies May Be Useful In Short Stature Patients With Nonspecific Skeletal Abnormalities Caused By Acan Heterozygous Mutations: Six Chinese Cases And Literature Review. *Endocr Pract* (2020) 26(11):1255–68. doi: 10.4158/ep-2019-0518
14. Liang H, Miao H, Pan H, Yang H, Chen S, Gong F, et al. Clinical Characteristics of Pseudoachondroplasia and Analysis of COMP Gene Mutation. *Chin J Endocrinol Metab* (2019) 35(12):1006–13. doi: 10.3760/cma.j.issn.1000-6699.2019.12.003

15. Ke X, Liang H, Miao H, Yang H, Wang L, Gong F, et al. Clinical Characteristics of Short-Stature Patients With an NPR2 Mutation and the Therapeutic Response to rhGH. *J Clin Endocrinol Metab* (2021) 106(2):431–41. doi: 10.1210/clinem/dgaa842
16. Zhang H, Yang R, Wang Y, Ye J, Han L, Qiu W, et al. A Pilot Study of Gene Testing of Genetic Bone Dysplasia Using Targeted Next-Generation Sequencing. *J Hum Genet* (2015) 60(12):769–76. doi: 10.1038/jhg.2015.112
17. Plachy L, Dusatkova P, Maratova K, Petruzelkova L, Elblova L, Kolouskova S, et al. Familial Short Stature-A Novel Phenotype of Growth Plate Collagenopathies. *J Clin Endocrinol Metab* (2021) 106(6):1742–9. doi: 10.1210/clinem/dgab084
18. Long F, Ornitz DM. Development of the Endochondral Skeleton. *Cold Spring Harb Perspect Biol* (2013) 5(1):a008334. doi: 10.1101/cshperspect.a008334
19. Ballock RT, O'Keefe RJ. Physiology and Pathophysiology of the Growth Plate. *Birth Defects Res C Embryo Today* (2003) 69(2):123–43. doi: 10.1002/bdrc.10014
20. Zhang B, Zhang Y, Wu N, Li J, Liu H, Wang J. Integrated Analysis of COL2A1 Variant Data and Classification of Type II Collagenopathies. *Clin Genet Mar* (2020) 97(3):383–95. doi: 10.1111/cge.13680
21. Carter EM, Raggio CL. Genetic and Orthopedic Aspects of Collagen Disorders. *Curr Opin Pediatrics* (2009) 21(1):46–54. doi: 10.1097/MOP.0b013e32832185c5
22. Rajpar MH, McDermott B, Kung L, Eardley R, Knowles L, Heeran M, et al. Targeted Induction of Endoplasmic Reticulum Stress Induces Cartilage Pathology. *PloS Genet* (2009) 5(10):e1000691. doi: 10.1371/journal.pgen.1000691
23. Blaschke UK, Eikenberry EF, Hulmes DJ, Galla HJ, Bruckner P. Collagen XI Nucleates Self-Assembly and Limits Lateral Growth of Cartilage Fibrils. *J Biol Chem* (2000) 275(14):10370–8. doi: 10.1074/jbc.275.14.10370
24. Czarny-Ratajczak M, Lohiniva J, Rogala P, Kozlowski K, Perälä M, Carter L, et al. A Mutation in COL9A1 Causes Multiple Epiphyseal Dysplasia: Further Evidence for Locus Heterogeneity. *Am J Hum Genet* (2001) 69(5):969–80. doi: 10.1086/324023
25. Vuoristo MM, Pappas JG, Jansen V, Ala-Kokko L. A Stop Codon Mutation in COL11A2 Induces Exon Skipping and Leads to non-Ocular Stickler Syndrome. *Am J Med Genet A* (2004) 130a(2):160–4. doi: 10.1002/ajmg.a.30111
26. Bateman JF, Freddi S, McNeil R, Thompson E, Hermanns P, Savarirayan R, et al. Identification of Four Novel COL10A1 Missense Mutations in Schmid Metaphyseal Chondrodysplasia: Further Evidence That Collagen X NC1 Mutations Impair Trimer Assembly. *Hum Mutat* (2004) 23(4):396. doi: 10.1002/humu.9222
27. Kong L, Shi L, Wang W, Zuo R, Wang M, Kang Q. Identification of Two Novel COL10A1 Heterozygous Mutations in Two Chinese Pedigrees With Schmid-Type Metaphyseal Chondrodysplasia. *BMC Med Genet* (2019) 20(1):200. doi: 10.1186/s12881-019-0937-1
28. Bonafe L, Cormier-Daire V, Hall C, Lachman R, Mortier G, Mundlos S, et al. Nosology and Classification of Genetic Skeletal Disorders: 2015 Revision. *Am J Med Genet A* (2015) 167a(12):2869–92. doi: 10.1002/ajmg.a.37365
29. Lindgren AC, Lindberg A. Growth Hormone Treatment Completely Normalizes Adult Height and Improves Body Composition in Prader-Willi Syndrome: Experience From KIGS (Pfizer International Growth Database). *Horm Res* (2008) 70(3):182–7. doi: 10.1159/000145019
30. Dahlgren J. GH Therapy in Noonan Syndrome: Review of Final Height Data. *Horm Res Dec* (2009) 72:46–8. doi: 10.1159/000243779
31. Toumba M, Albanese A, Azcona C, Stanhope R. Effect of Long-Term Growth Hormone Treatment on Final Height of Children With Russell-Silver Syndrome. *Horm Res Paediatr* (2010) 74(3):212–7. doi: 10.1159/000295924
32. Kanazawa H, Tanaka H, Inoue M, Yamanaka Y, Namba N, Seino Y. Efficacy of Growth Hormone Therapy for Patients With Skeletal Dysplasia. *J Bone Miner Metab* (2003) 21(5):307–10. doi: 10.1007/s00774-003-0425-7
33. Terhal PA, Nievelstein RJ, Verver EJ, Topsakal V, van Dommelen P, Hoornaert K, et al. A Study of the Clinical and Radiological Features in a Cohort of 93 Patients With a COL2A1 Mutation Causing Spondyloepiphyseal Dysplasia Congenita or a Related Phenotype. *Am J Med Genet A* (2015) 167a(3):461–75. doi: 10.1002/ajmg.a.36922
34. Pinto G, Cormier-Daire V, Le Merrer M, Samara-Boustani D, Baujat G, Fresneau L, et al. Efficacy and Safety of Growth Hormone Treatment in Children With Hypochondroplasia: Comparison With an Historical Cohort. *Horm Res Paediatr* (2014) 82(6):355–63. doi: 10.1159/000364807
35. Vieira NE, Marini JC, Hopkins E, Abrams SA, Yergey AL. Effect of Growth Hormone Treatment on Calcium Kinetics in Patients With Osteogenesis Imperfecta Type III and IV. *Bone* (1999) 25(4):501–5. doi: 10.1016/s8756-3282(99)00186-6
36. Antoniazzi F, Mottes M, Fraschini P, Brunelli PC, Tatò L. Osteogenesis Imperfecta: Practical Treatment Guidelines. *Paediatr Drugs* (2000) 2(6):465–88. doi: 10.2165/00128072-200002060-00005
37. Antoniazzi F, Monti E, Venturi G, Franceschi R, Doro F, Gatti D, et al. GH in Combination With Bisphosphonate Treatment in Osteogenesis Imperfecta. *Eur J Endocrinol* (2010) 163(3):479–87. doi: 10.1530/eje-10-0208

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Endocrinology

CORRECTION
published: 06 April 2022
doi: 10.3389/fendo.2022.896742

Corrigendum: Clinical Characteristics of Short-Stature Patients With Collagen Gene Mutation and the Therapeutic Response to rhGH

*Meiping Chen, Hui Miao, Hanting Liang, Xiaoan Ke, Hongbo Yang, Fengying Gong, Linjie Wang, Lian Duan, Shi Chen, Hui Pan and Huijuan Zhu**

Key Laboratory of Endocrinology of National Health Commission, Department of Endocrinology, State Key Laboratory of Complex Severe and Rare Diseases Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

Keywords: short stature, skeletal abnormalities, collagenopathies, next-generation sequencing, growth hormone treatment

A Corrigendum on

Clinical Characteristics of Short-Stature Patients With Collagen Gene Mutation and the Therapeutic Response to rhGH

by Chen M, Miao H, Liang H, Ke X, Yang H, Gong F, Wang L, Duan L, Chen S, Pan H and Zhu H. Front. Endocrinol. (2022) 13:820001. doi: 10.3389/fendo.2022.820001.

OPEN ACCESS

Approved by:
Frontiers Editorial Office, Frontiers Media SA, Switzerland

***Correspondence:**
Huijuan Zhu
shengxin2004@163.com

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *15 March 2022*
Accepted: *18 March 2022*
Published: *06 April 2022*

Citation:
Chen M, Miao H, Liang H, Ke X, Yang H, Gong F, Wang L, Duan L, Chen S, Pan H and Zhu H (2022) Corrigendum: Clinical Characteristics of Short-Stature Patients With Collagen Gene Mutation and the Therapeutic Response to rhGH. Front. Endocrinol. 13:896742. doi: 10.3389/fendo.2022.896742

In the **Results**, subsection "*Comparison of Collagen Gene-Related Short Stature With Other Short Stature Genes and Growth Response to rhGH Treatment*", paragraph 2 as published originally, **Table 4** was incorrectly cited. The following sentence "A total of 121 affected individuals diagnosed genetically are shown in **Table 3**." should have read "A total of 121 affected individuals diagnosed genetically are shown in **Table 4**."

Also, in the **Results**, subsection "*Comparison of Collagen Gene-Related Short Stature With Other Short Stature Genes and Growth Response to rhGH Treatment*", final paragraph, incorrect height Z scores were presented.

The sentences "In ACAN-related short stature, rhGH treatment significantly increased height and the height Z score (from -2.9 ± 1.0 to 0.6 ± 0.7) after 2.8 ± 0.4 years of administration. For NPR2-related short stature, height Z scores were significantly improved from 3.1 ± 0.8 to 2.0 ± 1.0 after 3.8 ± 0.6 years of treatment." should have read "In ACAN-related short stature, rhGH treatment significantly increased height and the height Z score (from -2.9 ± 1.0 to -2.2 ± 1.1) after 2.8 ± 0.4 years of administration. For NPR2-related short stature, height Z scores were significantly improved from -3.1 ± 0.8 to -2.0 ± 1.0 after 3.8 ± 0.6 years of treatment."

The authors apologize for these errors and state that they do not change the scientific conclusions of the article in any way. The original article has been updated.

frontiers in Endocrinology

MINI REVIEW
published: 18 February 2022
doi: 10.3389/fendo.2022.853290

Diagnosis of GH Deficiency Without GH Stimulation Tests

Anastasia Ibba and Sandro Loche *

Struttura Semplice Dipartimentale (SSD) Endocrinologia Pediatrica e Centro Screening Neonatale, Ospedale Pediatrico Microcitemico "A. Cao", Azienda di Rilievo Nazionale ed Alta Specializzazione (ARNAS) G. Brotzu, Cagliari, Italy

Growth hormone deficiency (GHD) is the most commonly affected pituitary hormone in childhood with a prevalence of 1 in 4000–10000 live births. GH stimulation testing (GHST) is commonly used in the diagnostic workup of GHD. However, GHD can be diagnosed in some clinical conditions without the need of GHST. The diagnosis of GHD in newborns does not require stimulation testing. Likewise infants/children with delayed growth and/or short stature associated with neuroradiological abnormalities and one or more additional pituitary hormone deficiencies may not need GHST. This review summarizes the current evidence on the diagnosis of GHD without stimulation tests.

OPEN ACCESS

Edited by:
M. Savage,
Queen Mary University of London, United Kingdom

Reviewed by:
Alan David Rogol,
University of Virginia, United States

***Correspondence:**
Sandro Loche
sandro.loche@aob.it

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *12 January 2022*
Accepted: *27 January 2022*
Published: *18 February 2022*

Citation:
Ibba A and Loche S (2022) Diagnosis of GH Deficiency Without GH Stimulation Tests. Front. Endocrinol. 13:853290. doi: 10.3389/fendo.2022.853290

Keywords: children, growth, growth hormone, IGF-1, growth hormone stimulation tests, newborn

INTRODUCTION

Growth hormone deficiency (GHD) has a prevalence of 1:4000-10000, most cases are isolated (IGHD) and the majority of them are idiopathic. GHD can also be combined with other pituitary hormone deficiencies (multiple pituitary hormone deficiency-MPHD). Both IGHD and MPHD can be congenital or acquired (tumours, trauma, brain infections, radiotherapy). Congenital IGHD can be due to genetic mutations in the genes encoding GH (GH1) or the GH-releasing hormone receptor (GHRHR) (1, 2). Mutations in the genes encoding transcription factors like SOX3, HESX1, GLI2, OTX2, LHX3, LHX4, PROP1, and POU1F1 usually cause MPHD (3) but, occasionally, GHD can be the only pituitary hormone deficiency (1, 2). GHD can develop at any age, so the signs and symptoms vary accordingly. GHD in newborns may present with hypoglycaemia, jaundice, or with underdeveloped male genitalia, whereas in older children it manifests primarily with short stature and/or decreased growth (4).

In the last decades the diagnosis of idiopathic IGHD has been the subject of intensive debate and it is still controversial. GH stimulation testing is not necessary in neonates and also in infants with a combination of clinical signs of GHD, low insulin-like growth factor 1 (IGF-1) and IGF binding protein-3 (IGFBP-3), MPHD and/or an abnormal brain magnetic resonance imaging (MRI) (5, 6).

The most recent guidelines (7) still recommend to perform GHSTs in children and adolescents with suspected GHD. However, some authors (8, 9) suggest that also in this age group the diagnosis of GHD may be based on a combination of auxological, biochemical (IGF-1 and IGFBP-3), neuroradiological and genetic findings and that GHSTs are not always necessary. It should be pointed out that the diagnosis of GHD is primarily auxologic (10, 11).

In this review we summarize the current evidence on the diagnosis of GHD without the use of GHSTs in the paediatric population.

PHYSIOPATHOLOGY

GH is a 191-amino acid protein synthesized, stored and secreted in a pulsatile manner by pituitary somatotroph cells. The synthesis and release of GH are under the control of various hormones, including GH-releasing hormone (GHRH), somatostatin, ghrelin, IGF-1, thyroid hormone, gonadal steroids and glucocorticoids. At birth and in the first week of life, GH levels are high and pulsatile, with elevated baseline, mean and peak levels (4) and rapidly decrease during the following weeks and increase with chronic malnutrition, chronic kidney disease, exercise, trauma and sepsis (12). GH plays a key role in glucose and fat metabolism in the newborn (4, 13), in increasing bone length and density in children and adolescents, but also in increasing muscle mass, regulating lipid and carbohydrates metabolism and body water throughout life. GH action is exerted directly on target tissues or indirectly by insulin growth factors (IGFs) (14). IGF-1, the main GH effector, is mostly secreted by the liver, and circulates bound to specific insulin growth factor binding protein (IGFBPs 1-6), mainly IGFBP-3. IGF-1 secretion is influenced also by malnutrition, thyroid hormone, sex hormones, chronic diseases and inflammation and anorexia nervosa (15–19). In contrast, IGF-1 values remain low for at least the first 15–18 months of age and increase until a pubertal peak (20, 21). Measurement of a random serum GH level is not helpful for the diagnosis of GHD except in some specific cases (see below). In fact, serum GH levels between normal pulses of GH secretion, are often low, below the limits of sensitivity of most conventional assays. For these reasons stimulation tests have been introduced in the diagnostic workup of GHD many years ago. A large number of GHSTs have been proposed in the last decades (6, 22–25). However, GHSTs are not physiological, have poor specificity and reproducibility (24), and cause a high number of false pathological responses. Furthermore, there are no age- and gender-related normative data, and the diagnostic cut-offs are often arbitrarily established. In addition, GH secretion is influenced by several factors (such obesity, undernutrition, puberty) and entails high costs and discomfort for the patients. Notwithstanding the above limitations, GHSTs are still used as the gold standard for the diagnosis of paediatric GHD (7, 26–29). These limitations are even more evident in children younger than 4 years, in which the accuracy of stimulation tests has not been formally addressed, and most of the currently used tests are burdened by side effects (5, 24). For all the above reasons the decision to perform a GHST should be well reasoned and based on the severity of short stature, height velocity, medical history and physical examination findings. The diagnostic GH peak cut-off is still matter of discussion between scientific societies, and so far it has been arbitrarily established by the single centre and currently ranges between 3 to 10 μg/L (6, 7, 11, 22, 24, 27, 30–32).

CLINICAL PRESENTATION OF GHD

Newborn

In consideration of the important metabolic role of GH in the neonatal period, the prompt identification of a newborn with GHD is crucial to start replacement treatment rapidly. GHD in neonates can be isolated but often presents as MPHD, and the clinical presentation and its severity depend on the number of affected hormones. Neonates might present non-specific symptoms and signs, such as lethargy and poor weight gain, or more specific life-threatening emergencies (33), including respiratory distress, apnoea, cyanosis, poor feeding, hypotonia, long-term cholestatic jaundice, severe hypoglycaemia with or without seizures, temperature dysregulation, electrolyte abnormalities, haemodynamic instability and/or neonatal sepsis. Other physical findings can suggest the presence of GHD such as eye abnormalities, microphallus, microphthalmia and single central maxillary incisor. Intrauterine growth is generally not affected by GHD, and birth weight and length are usually within normal limits, although slightly reduced.

Infant/Child

Short stature, defined as a height more than 2 SD below the population mean, or growth arrest/deceleration with normal/increased weight and delayed skeletal maturation may be the only signs of GHD in infancy and childhood. Diminished height velocity often precedes short stature. The typical GHD clinical phenotype in infants is persistent growth failure and/or short stature associated with truncal adiposity and micropenis, immature appearance, mid-facial hypoplasia, delayed dentition and frontal bossing with depressed nasal bridge.

Most cases of IGHD in childhood are idiopathic. However, pituitary masses, brain tumours, infections of central nervous system should always be ruled out. Furthermore, GHD should be suspected in short children who underwent cranial irradiation (34, 35) or suffered from brain injuries (36).

ESTABLISHING A DIAGNOSIS

Newborn

The neonatal period is characterized by high GH levels (hypersomatotropism of the newborn) (37, 38), which enable the diagnosis of GHD without the use of pharmacological stimulation (20, 39). Furthermore GHSTs are contraindicated under the age of 1-2 years (2). Reasons for this are primarily due to safety (GHSTs need a fasting period), to the amount of time needed, to the potential for hypoglycaemia, or other side effects depending on the GH secretagogue used.

A single low GH measurement is traditionally used to confirm the clinical suspicion of neonatal GHD. The sample is preferably taken during hypoglycaemic episodes (critical sample), in plasma, serum or newborn screening cards (39) within the first week of life. However, the specificity of a single GH measurement during spontaneous hypoglycaemia has been questioned, and some authors (40) do not deem it sufficient to diagnose GHD.

However, the observation of normal GH concentration can be useful to exclude GHD. Over the years, various cut-offs of GH as indicative of GH sufficiency in the newborn have been proposed ranging from 7 to 20 μg/L during an hypoglycaemic episode (20, 22). Recently, Binder et al. (39) demonstrated that GH concentration <7 μg/L in the newborn screening card confirms severe GHD with high accuracy in term newborns with a specific phenotype such as recurrent hypoglycaemia, additional pituitary hormone deficiencies and/or a significant hypothalamic-pituitary abnormality on cerebral MRI (**Table 1**). The use of newborn screening card still needs to be validated since its reliability has not been confirmed (41). According to current guidelines (7) the diagnosis of GHD in newborns is possible in the presence of GH concentrations ≤5 ng/mL in a newborn with additional pituitary hormone deficiencies or/and the triad of ectopic posterior pituitary, pituitary hypoplasia and abnormal pituitary stalk (**Table 1**).

Infant/Child

An accurate and early diagnosis is important for a prompt treatment initiation, essential to optimize child growth and adult height and to avoid co-morbidities such as impaired quality of life, bone and metabolic health (7, 26). Despite more than 50 years of paediatric hGH replacement, the ability to make a definitive diagnosis of GHD in children is still limited. The diagnosis of GHD is traditionally based on auxology and the lack of response to two different GHSTs, but it is not always straightforward (10).

In infants with history of hypoglycaemia, hyperbilirubinemia, poor growth, midline defects, microphallus, low IGF-1 and IGFBP-3, MPHD, such as TSH and ACTH deficiency, and/or an abnormal brain MRI, the diagnosis of GHD is possible without stimulation test (6) (**Table 1**).

According to recent guidelines (7) a diagnosis of GHD without GHSTs in children is suggested only in subjects that fulfil all the following criteria: auxological characteristics, presence of hypothalamic-pituitary defects (congenital or acquired), and one additional pituitary hormone deficiency (**Table 1**). However, according to some authors (26) there are more conditions in which GHSTs might be not necessary, such as in case of acquired GHD due to intracranial tumours, severe traumatic brain injury or cranial radiotherapy (**Table 2**). Given to the lack of sufficient evidence, the guidelines do not recommend establishing the diagnosis of GHD without GHSTs in patients with these conditions (7, 34, 35). Due to the low reliability of the GHSTs, alternative diagnostic strategies such as measurement of IGF-1 and IGFBP-3, genetic testing and neuroimaging have been considered over the years for the diagnosis of GHD in children (5, 8, 9, 42). In our opinion, patients with auxological characteristics associated with abnormal hypothalamic/pituitary morphology and low IGF-1 do not require GHST.

Recently Clément et al. (26) developed and validated an accurate clinical prediction rule for the diagnosis of GHD without GHSTs in children who meet the criteria required for GHSTs according to the guidelines (22), but with specific comorbidities such as the presence of pituitary dysgenesis on MRI or two or more anterior pituitary hormone deficiencies (**Table 2**).

However, recent guidelines (6) still consider the measurements of IGF-1, IGFBP-3 levels, brain MRI and genetic tests only as a support for the diagnosis.

IGF-1 and IGFBP-3

Measurement of IGF-1 is considered not useful in newborns since its levels remain low for at least the first 15–18 months of age and then progressively increase reaching a peak at mid-puberty (20, 21). The usefulness of IGF-1 measurement in children, alone or in combination with IGFBP-3, for the diagnosis of GHD has been the subject of a number of studies (7, 18, 23, 28, 30, 31, 42–50). The results of all these studies have been controversial, and their findings are hardly comparable because of the use of different assays, different measure unit (μg/ml, percentiles, SDS), as well as patients' selection (43). However, most studies showed poor sensitivity and specificity in the diagnosis of GHD in children and most authors concluded that IGF-1 measurement is useful for the diagnosis of GHD only when combined with auxological parameters and the results of GHSTs (31, 42, 44, 46, 51–53). IGF-1 levels should be interpreted taking into consideration age, gender, pubertal status and body mass index (18). Moreover reduced IGF-1 levels may be observed children with malnutrition (19), hypothyroidism, hepatic disease or diabetes mellitus and there is overlap between normal and GHD children. Therefore, although very low levels of IGF-1 are strongly suggestive of GHD, normal IGF-1 concentrations do not exclude GHD at any age (54). Wit et al. (18) recently proposed specific steps for the clinicians for the use of IGF-1 measurement to estimate the probability of GHD in a child with growth failure based on *pre-* and *post-test likelihood.* In our personal experience about 40% of patients with severe GHD have IGF-1 concentrations higher than -2 SDS, overlapping values found in non-GHD children (53).

IGFBP-3 levels have also been considered for the diagnosis of GHD since it is less influenced by nutrition than IGF-1.

TABLE 1 | Conditions in which it is not necessary to perform GHSTs according to current guidelines (6, 7, 22, 39).

Newborns	GH ≤ 7 μg/L during hypoglycaemia episode with a specific phenotype Random GH ≤ 5 ng/mL with additional pituitary hormone deficiencies or/and the triad of ectopic posterior pituitary, pituitary hypoplasia and abnormal stalk
Infants	Suggestive history of clinical GHD (short stature and/or low height velocity), low IGF-1 and IGFBP-3, multiple pituitary hormone deficiencies, and/or an abnormal cranial MRI
Children	Auxological characteristics (short stature and/or low height velocity), presence of hypothalamic-pituitary defects (congenital or acquired), and one additional pituitary hormone deficiency

TABLE 2 | Conditions in which it is not necessary to perform GHSTs. Modified from Clément et al. (26).

Auxological criteria required for performing GHSTs according to the Summary Statement of the Growth Hormone Research Society (22):	1) severe short stature, defined as a height more than 3 SD below the mean; 2) height more than 1.5 SD below the midparental height; 3) height more than 2 SD below the mean and a height velocity over 1 year more than 1 SD below the mean for chronological age, or a decrease in height SD of more than 0.5 over 1 year in children over 2 year of age; 4) in the absence of short stature, a height velocity more than 2 SD below the mean over 1 year or more than 1.5 SD sustained over 2 year; 5) signs indicative of an intracranial lesion; 6) signs of MPHD; 7) neonatal symptoms and signs of GHD.
PLUS	
Pituitary dysgenesis on MRI or Two or more anterior pituitary hormone deficiencies or At least one anterior pituitary hormone deficiency plus one of the following: a. Neonatal symptoms of pituitary deficiency (hypoglycaemia or hypogenitalism) b. Central diabetes insipidus c. Clinical or radiological craniofacial midline abnormalities d. Suprasellar or sellar tumor/surgery e. Cranial radiotherapy ≥18 Gy	

However, many studies have reported no advantage of measuring IGFBP-3 over IGF-1 (55).

Magnetic Resonance Imaging

The differential diagnosis of hypopituitarism has greatly improved thanks to diagnostic accuracy of MRI that has increased our knowledge of pituitary morphology and function (56, 57). Brain MRI with a focus on the pituitary and hypothalamus is essential during the initial evaluation of newborns with midline defects, microphallus, and hypoglycaemia. In a infant with a highly suspicious history of GHD, plus other pituitary hormone deficiencies, or neurologic abnormalities, the presence of an abnormal brain MRI allows the diagnosis of GHD without GHSTs (6). Abnormalities found on MRI that are more suggestive of GHD include the absence of the anterior pituitary gland (empty sella), an ectopic posterior pituitary gland, and hypoplasia/absence of the pituitary stalk and/or pituitary gland (58). However the presence of a small pituitary gland by itself is not sufficient to make the diagnosis of GHD, but it may suggest the need for a more extensive evaluation of pituitary function (6). In children younger than 4 years, MRI has been proposed as first-line investigation (5) in order to reduce cost/benefit ratio and allow earlier start of treatment, and to postpone GHSTs to an age when they can be more easily performed and interpreted. Neuroimaging in association with IGF-1 assessment has been proposed about 20 years ago in children with clinical suspicion of GHD as an alternative to GHSTs (8). However the current guidelines (6) still recommend to perform MRI of the hypothalamus and pituitary after the diagnosis of GHD is confirmed by GHSTs. Therefore, if GHD has been excluded by GHSTs, MRI is typically not indicated.

Genetic Testing

Genetic and/or epigenetic testing is not required for all suspects of GHD but it is suggested in the diagnostic assessment of a patient whose phenotype suggests a high likelihood of a genetic cause (6) such as in case of suspected congenital hypopituitarism, early onset of growth failure, positive family history, height more than 3 SD below the mean, extremely low GH response to GHSTs and, very low IGF-1 and IGFBP-3 levels. The most common mutations in patients with isolated GHD have been identified in GH1 and GHRHR genes and may be associated with a normal MRI scan. Other gene mutations (i.e. POU1F1, PROP1, LHX3, LHX4, HESX1, SOX2, SOX3, etc.) are generally associated with MPHD (2), and present with typical clinical and neuroradiological features. With a greatly use of genetic testing it is possible that other conditions may include GHD in the differential diagnosis (59).

CONCLUSIONS

In most cases of suspected GHD current guidelines still recommend the use of GHSTs plus auxological criteria. However, GHSTs are not accurate, and in some instances a diagnosis can be made based on other clinical, laboratory, genetic and neuroimaging evaluation. IGF-1 and IGFBP-3 measurement have high specificity but low sensitivity and thus normal concentrations do not exclude GHD at any age. MRI of the hypothalamic–pituitary region might be helpful in identifying GHD when associated with other cerebral abnormalities, and genetic testing can provide definitive diagnosis in some selected patients. The diagnosis of GHD may be straightforward in neonates, infants and children with organic lesions, irradiation or trauma, but is still puzzling in all other conditions, requiring careful clinical, laboratory and imaging investigation.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the design and writing of the review. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Alatzoglou KS, Webb EA, Le Tissier P, Dattani MT. Isolated Growth Hormone Deficiency (GHD) in Childhood and Adolescence: Recent Advances. *Endocrine Rev* (2014) 35:376–432. doi: 10.1210/er.2013-1067
2. Bosch i Ara L, Katugampola H, Dattani MT. Congenital Hypopituitarism During the Neonatal Period: Epidemiology, Pathogenesis, Therapeutic Options, and Outcome. *Front Pediatr* (2021) 8:600962. doi: 10.3389/fped.2020.600962
3. Alatzoglou KS, Dattani MT. Genetic Forms of Hypopituitarism and Their Manifestation in the Neonatal Period. *Early Hum Dev* (2009) 85:705–12. doi: 10.1016/j.earlhumdev.2009.08.057
4. Ogilvy-Stuart AL. Growth Hormone Deficiency (GHD) From Birth to 2 Years of Age: Diagnostic Specifics of GHD During the Early Phase of Life. *Horm Res Paediatr* (2003) 60:2–9. doi: 10.1159/000071219
5. Pampanini V, Pedicelli S, Gubinelli J, Scirè G, Cappa M, Boscherini B, et al. Brain Magnetic Resonance Imaging as First-Line Investigation for Growth Hormone Deficiency Diagnosis in Early Childhood. *Horm Res Paediatr* (2015) 84:323–30. doi: 10.1159/000439590
6. Collett-Solberg PF, Ambler G, Backeljauw PF, Bidlingmaier M, Biller BMK, Boguszewski MCS, et al. Diagnosis, Genetics, and Therapy of Short Stature in Children: A Growth Hormone Research Society International Perspective. *Horm Res Paediatr* (2019) 92:1–14. doi: 10.1159/000502231
7. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, et al. Drug and Therapeutics Committee and Ethics Committee of the Pediatric Endocrine Society. Guidelines for Growth Hormone and Insulin-Like Growth Factor-I Treatment in Children and Adolescents: Growth Hormone Deficiency, Idiopathic Short Stature, and Primary Insulin-Like Growth Factor-I Deficiency. *Horm Res Paediatr* (2016) 86:361–97. doi: 10.1159/000452150
8. Badaru A, Wilson D. Alternatives to Growth Hormone Stimulation Testing in Children. *Trends Endocrinol Metab* (2004) 15:252–8. doi: 10.1016/j.tem.2004.06.004
9. Tenenbaum-Rakover Y, Hujeirat Y, Admoni O, Khayat M, Allon-Shalev S, Hess O. Can Auxology, IGF-I and IGFBP-3 Measurements Followed by MRI and Genetic Tests Replace GH Stimulation Tests in the Diagnosis of GH Deficiency in Children? *J Pediatr Endocrinol Metab* (2010) 23(4):387–94. doi: 10.1515/jpem.2010.060
10. Rosenfeld RG, Albertsson-Wikland K, Cassorla F, Frasier SD, Hasegawa Y, Hintz RL, et al. Diagnostic Controversy: The Diagnosis of Childhood Growth Hormone Deficiency Revisited. *J Clin Endocrinol Metab* (1995) 80:1532–40. doi: 10.1210/jcem.80.5.7538145
11. Rosenfeld RG. Is Growth Hormone Deficiency a Viable Diagnosis? *J Clin Endocrinol Metab* (1997) 82:349–51. doi: 10.1210/jcem.82.2.3841. D. M.
12. Møller N, Jørgensen JOL. Effects of Growth Hormone on Glucose, Lipid, and Protein Metabolism in Human Subjects. *Endocrine Rev* (2009) 30:152–77. doi: 10.1210/er.2008-0027
13. Mamilly L, Pyle-Eilola AL, Chaudhari M, Henry RK. The Utility of a Random Growth Hormone Level in Determining Neonatal Growth Hormone Sufficiency. *Clin Endocrinol* (2021) 94:392–8. doi: 10.1111/cen.14364
14. Dehkhoda F, Lee CMM, Medina J, Brooks AJ. The Growth Hormone Receptor: Mechanism of Receptor Activation, Cell Signaling, and Physiological Aspects. *Front Endocrinol* (2018) 9:35. doi: 10.3389/fendo.2018.00035
15. Purandare A, Co Ng L, Godil M, Ahnn SH, Wilson TA. Effect of Hypothyroidism and Its Treatment on the IGF System in Infants and Children. *J Pediatr Endocrinol Metab* (2003) 16(1):35–42. doi: 10.1515/JPEM.2003.16.1.35
16. Jansson U, Kristiansson B, Magnusson P, Larsson L, Albertsson-Wikland K, Bjarnason R. The Decrease of IGF-I, IGF-Binding Protein-3 and Bone Alkaline Phosphatase Isoforms During Gluten Challenge Correlates With Small Intestinal Inflammation in Children With Coeliac Disease. *Eur J Endocrinol* (2001) 144(2):417–23. doi: 10.1530/eje.0.1440417
17. Støving RK, Hangaard J, Hagen C, Flyvbjerg A. Low Levels of the 150-kD Insulin-Like Growth Factor Binding Protein 3 Ternary Complex in Patients With Anorexia Nervosa: Effect of Partial Weight Recovery. *Horm Res Paediatr* (2003) 60:43–8. doi: 10.1159/000070826
18. Wit JM, Bidlingmaier M, de Bruin C, Oostdijk W. A Proposal for the Interpretation of Serum IGF-I Concentration as Part of Laboratory Screening in Children With Growth Failure. *Jcrpe* (2020) 12:130–9. doi: 10.4274/jcrpe.galenos.2019.2019.0176
19. Hawkes CP, Grimberg A. Insulin-Like Growth Factor-I Is a Marker for the Nutritional State. *Pediatr Endocrinol Rev* (2015) 13:499–511.
20. Binder G, Weidenkeller M, Blumenstock G, Langkamp M, Weber K, Franz AR. Rational Approach to the Diagnosis of Severe Growth Hormone Deficiency in the Newborn. *J Clin Endocrinol Metab* (2010) 95:2219–26. doi: 10.1210/jc.2009-2692
21. Bidlingmaier M, Friedrich N, Emeny RT, Spranger J, Wolthers OD, Roswall J, et al. Reference Intervals for Insulin-Like Growth Factor-1 (IGF-I) From Birth to Senescence: Results From a Multicenter Study Using a New Automated Chemiluminescence IGF-I Immunoassay Conforming to Recent International Recommendations. *J Clin Endocrinol Metab* (2014) 99:1712–21. doi: 10.1210/jc.2013-3059
22. Growth Hormone Research Society. Consensus Guidelines for the Diagnosis and Treatment of Growth Hormone (GH) Deficiency in Childhood and Adolescence: Summary Statement of the GH Research Society. *J Clin Endocrinol Metab* (2000) 85:3990–3. doi: 10.1210/jcem.85.11.6984.
23. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, et al. Consensus Statement on the Diagnosis and Treatment of Children With Idiopathic Short Stature: A Summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab* (2008) 93:4210–7. doi: 10.1210/jc.2008-0509
24. Binder G, Reinehr T, Ibáñez L, Thiele S, Linglart A, Woelfle J, et al. GHD Diagnostics in Europe and the US: An Audit of National Guidelines and Practice. *Horm Res Paediatr* (2019) 92:150–6. doi: 10.1159/000503783
25. Juul A, Bernasconi S, Clayton PE, Kiess W, DeMuinck-Keizer Schrama S. European Audit of Current Practice in Diagnosis and Treatment of Childhood Growth Hormone Deficiency. *Horm Res Paediatr* (2002) 58:233–41. doi: 10.1159/000066265
26. Clément F, Grinspon RP, Yankelevich D, Martín Benítez S, de la Ossa Salgado MC, Ropelato MG, et al. Development and Validation of a Prediction Rule for Growth Hormone Deficiency Without Need for Pharmacological Stimulation Tests in Children With Risk Factors. *Front Endocrinol* (2021) 11:624684. doi: 10.3389/fendo.2020.624684
27. Kim JH, Chae HW, Chin SO, Ku CR, Park KH, Lim DJ, et al. Diagnosis and Treatment of Growth Hormone Deficiency: A Position Statement From Korean Endocrine Society and Korean Society of Pediatric Endocrinology. *Endocrinol Metab* (2020) 35:272–87. doi: 10.3803/EnM.2020.35.2.272
28. Felício JS, Janaú LC, Moraes MA, Zahalan NA, de Souza Resende F, de Lemos MN, et al. Diagnosis of Idiopathic GHD in Children Based on Response to rhGH Treatment: The Importance of GH Provocative Tests and IGF-1. *Front Endocrinol* (2019) 10:638. doi: 10.3389/fendo.2019.00638
29. Murray PG, Dattani MT, Clayton PE. Controversies in the Diagnosis and Management of Growth Hormone Deficiency in Childhood and Adolescence. *Arch Dis Childhood* (2016) 101:96–100. doi: 10.1136/archdischild-2014-307228
30. Clemmons DR. Consensus Statement on the Standardization and Evaluation of Growth Hormone and Insulin-Like Growth Factor Assays. *Clin Chem* (2011) 57:555–9. doi: 10.1373/clinchem.2010.150631
31. Binder G, Huller E, Blumenstock G, Schweizer R. Auxology-Based Cut-Off Values for Biochemical Testing of GH Secretion in Childhood. *Growth Hormone IGF Res* (2011) 21:212–8. doi: 10.1016/j.ghir.2011.05.007
32. Guzzetti C, Ibba A, Pilia S, Beltrami N, Di Iorgi N, Rollo A, et al. Cut-Off Limits of the Peak GH Response to Stimulation Tests for the Diagnosis of GH Deficiency in Children and Adolescents: Study in Patients With Organic GHD. *Eur J Endocrinol* (2016) 175:41–7. doi: 10.1530/EJE-16-0105
33. Sobrier M-L, Maghnie M, Vié-Luton M-P, Secco A, di Iorgi N, Lorini R, et al. Novel HESX1 Mutations Associated With a Life-Threatening Neonatal Phenotype, Pituitary Aplasia, But Normally Located Posterior Pituitary and No Optic Nerve Abnormalities. *J Clin Endocrinol Metab* (2006) 91:4528–36. doi: 10.1210/jc.2006-0426
34. Pollock NI, Cohen LE. Growth Hormone Deficiency and Treatment in Childhood Cancer Survivors. *Front Endocrinol* (2021) 12:745932. doi: 10.3389/fendo.2021.745932

35. Sklar CA, Antal Z, Chemaitilly W, Cohen LE, Follin C, Meacham LR, et al. Hypothalamic–Pituitary and Growth Disorders in Survivors of Childhood Cancer: An Endocrine Society* Clinical Practice Guideline. *J Clin Endocrinol Metab* (2018) 103:2761–84. doi: 10.1210/jc.2018-01175
36. Dassa Y, Crosnier H, Chevignard M, Viaud M, Personnier C, Flechtner I, et al. Pituitary Deficiency and Precocious Puberty After Childhood Severe Traumatic Brain Injury: A Long-Term Follow-Up Prospective Study. *Eur J Endocrinol* (2019) 180:281–90. doi: 10.1530/EJE-19-0034
37. Laron Z, Mannheimer S, Pertzelan A, Nitzan M. Serum Growth Hormone Concentration in Full Term Infants. *Isr J Med Sci* (1966) 2:770–3.
38. Cornblath M, Parker ML, Reisner SH, Forbes AE, Daughaday WH. Secretion and Metabolism of Growth Hormone in Premature and Full-Term Infants1. *J Clin Endocrinol Metab* (1965) 25:209–18. doi: 10.1210/jcem-25-2-209
39. Binder G, Weber K, Rieflin N, Steinruck L, Blumenstock G, Janzen N, et al. Diagnosis of Severe Growth Hormone Deficiency in the Newborn. *Clin Endocrinol* (2020) 93(3):305–11. doi: 10.1111/cen.14264
40. Kelly A, Tang R, Becker S, Stanley CA. Poor Specificity of Low Growth Hormone and Cortisol Levels During Fasting Hypoglycemia for the Diagnoses of Growth Hormone Deficiency and Adrenal Insufficiency. *PEDIATRICS* (2008) 122:e522–8. doi: 10.1542/peds.2008-0806
41. Dominguez-Menéndez G, Cifuentes L, González C, Lagos M, Quiroga T, Rumié H, et al. Hormona De Crecimiento En Sangre De Papel Filtro Para El Diagnóstico De Deficiencia De Hormona De Crecimiento. *Rev Chil Pediatr* (2019) 90:145. doi: 10.32641/rchped.v90i2.674
42. Cianfarani S, Tondinelli T, Spadoni GL, Scire G, Boemi S, Boscherini B. Height Velocity and IGF-I Assessment in the Diagnosis of Childhood Onset GH Insufficiency: Do We Still Need a Second GH Stimulation Test? *Clin Endocrinol* (2002) 57:161–7. doi: 10.1046/j.1365-2265.2002.01591.x
43. Shen Y, Zhang J, Zhao Y, Yan Y, Liu Y, Cai J. Diagnostic Value of Serum IGF-1 and IGFBP-3 in Growth Hormone Deficiency: A Systematic Review With Meta-Analysis. *Eur J Pediatr* (2015) 174:419–27. doi: 10.1007/s00431-014-2406-3
44. Study Group on Physiopathology of growth processes and Council of ISPED, Federico G, Street ME, Maghnie M, Caruso-Nicoletti M, et al. Assessment of Serum IGF-I Concentrations in the Diagnosis of Isolated Childhood-Onset GH Deficiency: A Proposal of the Italian Society for Pediatric Endocrinology and Diabetes (SIEDP/ISPED). *J Endocrinological Invest* (2006) 29:732–7. doi: 10.1007/BF03344184
45. Wang Y, Zhang H, Cao M, Kong L, Ge X. Analysis of the Value and Correlation of IGF−1 With GH and IGFBP−3 in the Diagnosis of Dwarfism. *Exp Ther Med* (2019) 17(5):3689–93. doi: 10.3892/etm.2019.7393
46. Boquete HR, Sobrado PGV, Fideleff HL, Sequera AM, Giaccio AV, Suárez MG, et al. Evaluation of Diagnostic Accuracy of Insulin-Like Growth Factor (IGF)-I and IGF-Binding Protein-3 in Growth Hormone-Deficient Children and Adults Using ROC Plot Analysis. *J Clin Endocrinol Metab* (2003) 88:4702–8. doi: 10.1210/jc.2003-030412
47. Bussières L, Souberbielle J-C, Pinto G, Adan L, Noel M, Brauner R. The Use of Insulin-Like Growth Factor 1 Reference Values for the Diagnosis of Growth Hormone Deficiency in Prepubertal Children: IGF-1 Reference Values. *Clin Endocrinol* (2000) 52:735–9. doi: 10.1046/j.1365-2265.2000.00999.x
48. Cianfarani S, Liguori A, Germani D. IGF-I and IGFBP-3 Assessment in the Management of Childhood Onset Growth Hormone Deficiency. In: S Cianfarani, DR Clemmons, MO Savage, editors. *Endocrine Development*. Basel: KARGER (2005). p. 66–75. doi: 10.1159/000085757
49. Bogazzi F, Manetti L, Lombardi M, Giovannetti C, Raffaelli V, Urbani C, et al. Impact of Different Cut-Off Limits of Peak GH After GHRH-Arginine Stimulatory Test, Single IGF1 Measurement, or Their Combination in Identifying Adult Patients With GH Deficiency. *Eur J Endocrinol* (2011) 164:685–93. doi: 10.1530/EJE-10-1068
50. Inoue-Lima TH, Vasques GA, Scalco RC, Nakaguma M, Mendonca BB, Arnhold IJP, et al. IGF-1 Assessed by Pubertal Status has the Best Positive Predictive Power for GH Deficiency Diagnosis in Peripubertal Children. *J Pediatr Endocrinol Metab* (2019) 32:173–9. doi: 10.1515/jpem-2018-0435
51. Federico G, Cianfarani S. Usefulness of Serum Insulin-Like Growth Factor I Assessment in the Diagnosis of Childhood-Onset Growth Hormone Deficiency. *Hormone Res Paediatrics* (2010) 74:145–8. doi: 10.1159/000314895
52. Alawneh H, Khaledi O, Maita J. Insulin Like Growth Factor 1 as an Indicator of Growth Hormone Deficiency. *JRMS* (2015) 22:13–7. doi: 10.12816/0011355
53. Ibba A, Corrias F, Guzzetti C, Casula L, Salerno M, di Iorgi N, et al. IGF1 for the Diagnosis of Growth Hormone Deficiency in Children and Adolescents: A Reappraisal. *Endocrine Connections* (2020) 9:1095–102. doi: 10.1530/EC-20-0347
54. Cianfarani S, Liguori A, Boemi S, Maghnie M, Iughetti L, Wasniewska M, et al. Inaccuracy of Insulin-Like Growth Factor (IGF) Binding Protein (IGFBP)-3 Assessment in the Diagnosis of Growth Hormone (GH) Deficiency From Childhood to Young Adulthood: Association to Low GH Dependency of IGF-II and Presence of Circulating IGFBP-3 18-Kilodalton Fragment. *J Clin Endocrinol Metab* (2005) 90:6028–34. doi: 10.1210/jc.2005-0721
55. Phillip M, Chalew SA, Kowarski AA, Stene MA. Plasma IGFBP-3 and Its Relationship With Quantitative Growth Hormone Secretion in Short Children. *Clin Endocrinol* (1993) 39:427–32. doi: 10.1111/j.1365-2265.1993.tb02389.x
56. Hage C, Gan H-W, Ibba A, Patti G, Dattani M, Loche S, et al. Advances in Differential Diagnosis and Management of Growth Hormone Deficiency in Children. *Nat Rev Endocrinol* (2021) 17:608–24. doi: 10.1038/s41574-021-00539-5
57. Iorgi ND, Allegri AEM, Napoli F, Bertelli E, Olivieri I, Rossi A, et al. The Use of Neuroimaging for Assessing Disorders of Pituitary Development: Assessing Disorders of Pituitary Development. *Clin Endocrinol* (2012) 76:161–76. doi: 10.1111/j.1365-2265.2011.04238.x
58. Kalina MA, Kalina-Faska B, Gruszczyńska K, Baron J, Małecka-Tendera E. Usefulness of Magnetic Resonance Findings of the Hypothalamic-Pituitary Region in the Management of Short Children With Growth Hormone Deficiency: Evidence From a Longitudinal Study. *Childs Nerv Syst* (2012) 28:121–7. doi: 10.1007/s00381-011-1594-7
59. Wit JM, Oostdijk W, Losekoot M, van Duyvenvoorde HA, Ruivenkamp CAL, Kant SG. Mechanisms in Endocrinology: Novel Genetic Causes of Short Stature. *Eur J Endocrinol* (2016) 174:R145–73. doi: 10.1530/EJE-15-0937

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 18 February 2022
doi: 10.3389/fendo.2022.812568

Pubertal Timing and Growth Dynamics in Children With Severe Primary IGF-1 Deficiency: Results From the European Increlex® Growth Forum Database Registry

Peter Bang[1], Michel Polak[2], Valérie Perrot[3], Caroline Sert[3], Haris Shaikh[4] and Joachim Woelfle[5] on behalf of Eu-IGFD Registry Study Group*

[1] Division of Paediatrics, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden, [2] Paediatric Endocrinology, Gynaecology and Diabetology, Centre de Référence des Maladies Endocriniennes Rares de la Croissance, Hôpital Universitaire Necker Enfants Malades, AP-HP, Université de Paris, Paris, France, [3] Ipsen Pharma, Boulogne-Billancourt, France, [4] Ipsen Pharma, Milton Park, United Kingdom, [5] Endocrinology and Diabetology, University Children's Hospital, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

OPEN ACCESS

Edited by:
Madhusmita Misra, Massachusetts General Hospital and Harvard Medical School, United States

Reviewed by:
M. Savage, Queen Mary University of London, United Kingdom
Alan David Rogol, University of Virginia, United States

***Correspondence:**
Peter Bang
peter.bang@liu.se

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *10 November 2021*
Accepted: *20 January 2022*
Published: *18 February 2022*

Citation:
Bang P, Polak M, Perrot V, Sert C, Shaikh H and Woelfle J (2022) Pubertal Timing and Growth Dynamics in Children With Severe Primary IGF-1 Deficiency: Results From the European Increlex® Growth Forum Database Registry. Front. Endocrinol. 13:812568. doi: 10.3389/fendo.2022.812568

Background: Puberty is delayed in untreated children and adolescents with severe primary IGF-1 deficiency (SPIGFD); to date, it has not been reported whether recombinant human insulin-like growth factor-1 mecasermin (rhIGF-1) treatment affects this. Pubertal growth outcomes were extracted from the European Increlex® Growth Forum Database (Eu-IGFD) Registry (NCT00903110).

Methods: The Eu-IGFD Registry includes children and adolescents aged 2 to 18 years with growth failure associated with SPIGFD who are treated with rhIGF-1. Reported outcomes include: age at last registration of Tanner stage 1 and first registration of Tanner stage 2-5 (T2-T5; based on breast development for girls and genital development for boys, respectively); maximum height velocity during each Tanner stage; and pubertal peak height velocity (PPHV). Data cut-off was 13 May 2019.

Results: This analysis included 213 patients (132 boys and 81 girls). Mean (SD) age at last registration of T1 and first registration of T5 was 13.0 (2.0) and 16.3 (1.6) years, respectively, in boys and 11.6 (1.8) and 14.7 (1.5) years, respectively, in girls. Among patients reaching the end of puberty (25 boys and 11 girls), mean (SD) height SDS increased from -3.7 (1.4) at baseline in the Eu-IGFD Registry to -2.6 (1.4) at T5 in boys and from -3.1 (1.1) to -2.3 (1.5) in girls. Maximum height velocity was observed during T2 in girls and T3 in boys. Median (range) PPHV was 8.0 (0.3–13.0) cm/year in boys and 6.8 (1.3–9.6) cm/year in girls and occurred most frequently during T2. Overall, the adverse events seen in this analysis were in line with the known safety profile of rhIGF-1.

Conclusion: Children and adolescents treated with rhIGF-1 for SPIGFD with growth failure experienced an increase in height SDS in prepubertal years compared with baseline. Despite 1.5 years delay in pubertal start and a delayed and slightly lower PPHV, height SDS gain during puberty was maintained.

Keywords: growth retardation, severe primary insulin-like growth factor-1 deficiency, puberty, mecasermin, Eu-IGFD Registry

INTRODUCTION

The growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis is crucial for linear growth and pubertal growth promotion (1, 2), and IGF-1 deficiency causes severe growth retardation. Severe primary IGF-1 deficiency (SPIGFD) comprises a group of rare growth disorders, with a prevalence of approximately 1% within the spectrum of IGF-1 deficiency disorders (3). The GH/IGF-1 axis is disrupted in children with SPIGFD with GH insensitivity (low IGF-1 levels, despite normal or elevated GH secretion) (4, 5), leading to growth failure. Physical characteristics resulting from SPIGFD include extremely short stature, retarded organ growth, small hands and feet, and underdevelopment and weakness of the muscular system (6, 7). Disruption to the GH/IGF-1 axis is also thought to have a negative impact on gonadal function and pubertal development in patients with SPIGFD (8–11).

Growth failure associated with SPIGFD in children aged 2 to 18 years can be successfully treated, especially when diagnosed early (12), with long-term administration of recombinant human IGF-1 (rhIGF-1). The rhIGF-1 Increlex® (mecasermin [rDNA origin]; Ipsen Pharma, France) has been licensed for the treatment of SPIGFD since 2005 in the USA and 2007 in Europe (4, 5). Clinical trials have demonstrated that rhIGF-1 stimulates linear growth in children with SPIGFD, leading to increased height velocity (13, 14). Despite being used in clinical practice for over a decade, the impact of rhIGF-1 treatment on pubertal growth dynamics has not been extensively assessed. The effect of rhIGF-1 on pubertal development was described as part of a study assessing the safety and efficacy of rhIGF-1 in children with short stature and low IGF-1 levels, which showed that pubertal development occurred at appropriate ages in all individuals, except one; however, patient numbers in this study were low (15). Two important characteristics of the growth spurt at puberty are the pubertal peak height velocity (PPHV) and the age at which the PPHV occurs (16, 17); as the effect of rhIGF-1 treatment on these variables is currently unknown, and further research is required.

The European Increlex® Growth Forum Database (Eu-IGFD) Registry is an ongoing, open-label observational study to monitor the long-term safety and effectiveness of rhIGF-1 treatment in children and adolescents with growth failure in routine clinical practice. The Registry aims to monitor patients during, and after the end of treatment and to the attainment of near adult height (1). Here, we describe pubertal growth dynamics in children and adolescents with SPIGFD with growth failure who were treated with mecasermin and whose data were entered into the Eu-IGFD.

MATERIALS AND METHODS

Trial Design

The Eu-IGFD Registry is a descriptive, multicentre, observational, prospective, open-ended, non-interventional, post-authorization surveillance study (ClinicalTrials.gov ID: NCT00903110) conducted in ten European countries (Austria, Belgium, France, Germany, Italy, the Netherlands, Poland, Spain, Sweden and the UK), and initiated in December 2008.

The primary objective of the Eu-IGFD Registry is to collect long-term safety data on the use of mecasermin (rhIGF-1) for the treatment of children and adolescents with growth failure, including SPIGFD. The main secondary objective is to collect long-term effectiveness data for rhIGF-1 treatment in children and adolescents with growth failure. The Registry design has been described previously (1). The analysis presented in this manuscript covers data collected up to 13 of May 2019 and focuses on the effect of rhIGF-1 treatment on pubertal growth dynamics.

Patients

The Eu-IGFD Registry includes children and adolescents aged 2 to 18 years. All children and adolescents presenting at participating centres with growth failure associated with SPIGFD, for whom rhIGF-1 is indicated, and those who are already receiving treatment with rhIGF-1, are eligible for enrolment into the Registry and are assessed throughout their course of treatment (irrespective of subsequent treatment changes). The decision to prescribe rhIGF-1 treatment is made independently of the decision to enrol the patient into the Registry (1). Children and adolescents currently participating in either a mecasermin clinical trial or in any clinical trial for treatment of growth retardation were excluded from the Eu-IGFD Registry.

The analysis presented in this manuscript includes children and adolescents who were prepubertal (Tanner stage [T] 1; before breast development in girls and genital development in boys) at first rhIGF-1 intake in the Eu-IGFD Registry, were not receiving gonadotropin-releasing hormone (GnRH) agonist treatment and whose data were entered in the Eu-IGFD Registry before 13 May 2019.

Treatment

The administered dose of rhIGF-1 was in accordance with the European Summary of Product Characteristics (SmPC) for mecasermin (4) and local clinical practice. Dosing was individualised based on the treating-physician's clinical judgment. According to the mecasermin prescribing information, doses of 0.04–0.12 mg/kg bodyweight are given twice daily by subcutaneous injection before or shortly after a meal or snack. The timing and dose of rhIGF-1 treatment were at the discretion of the treating-physician and were independent of the decision to include patient data in the Eu-IGFD Registry.

Outcomes

Anonymous data in the patients' medical records are collected using an electronic case report form. General methodology for the Eu-IGFD Registry and information collected at baseline (or the visit closest to the start of rhIGF-1 treatment) and each follow-up visit has been described previously (1). The number and frequency of follow-up visits are determined by the investigator's judgment based on clinical need and mecasermin SmPC recommendations (1).

The following endpoints are reported in this manuscript: breast development in girls and genital development in boys were assessed at each visit according to Tanner stage, and the last registration of T1 and first registration of stages T2 to T5 were identified. Data were collected until patients reached adult height. In reporting the data on Tanner stage, an informal comparison was made with reference data from a healthy population in Denmark (18). Pubertal duration was defined as the time between last registration of T1 to first registrations of T4/T5. Maximum height velocity during each Tanner stage was calculated. PPHV was defined as the maximum annualised height velocity between two visits ≥6 months apart during T2 to T4/T5. The Tanner stage at which PPHV occurred was noted. The evolution in height SDS during pubertal development was also assessed.

Adverse events (AEs) were reported by the investigator and classified as serious or non-serious, as mild, moderate or severe, and whether they were related or not to rhIGF-1 treatment. Neoplasia events and all 'targeted' AEs were collected. Targeted AEs are defined as those AEs that were shown to occur frequently or historically associated with rhIGF-1 treatment (i.e., headache, otitis media, papilledema, hypoglycaemia, acromegalic facial changes, oedema, gynaecomastia, hearing loss, intracranial hypertension, lipohypertrophy at injection site, myalgia, sleep apnoea, tonsillar hypertrophy, and cardiomegaly).

Statistics

Descriptive statistics were used for all endpoints. Results are presented as mean (standard deviation [SD] or two-sided 95% confidence interval [CI] of the mean) and median (range or 25th and 75th percentiles). For categorical variables, the 95% CIs of the proportion are provided. Unless specified, continuous variables are given as median (range).

RESULTS

Patient Characteristics

Between December 2008 and May 2019, 281 patients were enrolled in the Eu-IGFD Registry; of these: 213 (132 boys and 81 girls) were prepubertal and were included in this analysis (**Table 1**); 157 (73.7%) were treatment-naïve (i.e., had not received previous growth-promoting treatment); and SPIGFD was the diagnosis in 188 (88.3%) patients (**Table 1**). All patients who were pubertal at the start of rhIGF-1 treatment in the Registry have been excluded from this analysis.

Of the 36 participants assessed until the end of puberty (excluding patients treated with gonadotropin-releasing hormone agonist), 14 were non-naïve, including 11 who were previously treated with rhGH, 2 who were previously treated with rhIGF-1, and 1 who was previously treated with both rhGH and rhIGF-1.

The median (range) duration of follow-up from the start of rhIGF-1 treatment was 4.3 (0.2–11.0) years. Mean rhIGF-1 doses remained stable as puberty progressed, with median doses of 120 μg/kg bid at all stages of puberty. At 1 year after initiation of rhIGF-1 treatment (after the titration period), 107 (50.2%) were receiving 120 μg/kg bid (the recommended maximum dose) (4) or above (only 6 patients were receiving a dose above 120μg/kg bid), 24 (11.3%) were receiving 100-120 μg/kg bid, and 82 (38.5%) were receiving <100 μg/kg bid.

Puberty and Pubertal Growth Dynamics

The mean (SD) age at start of rhIGF-1 treatment for patients reaching the end of puberty was 10.9 (2.56) years for boys, and 9.1 (1.83) years for girls. The mean age of patients at entry into each Tanner stage is shown in **Figure 1** (not all children had visits at every Tanner stage as the time between clinic visits varied). Compared with a Danish reference population of healthy children (18), boys and girls with SPIGFD had delayed entry into T2, with approximately 1.5 years, and with less delayed entry into T4/T5. Among the patients reaching the end of puberty (T5; 25 boys and 11 girls), mean (SD) pubertal duration from last T1 was 3.7 (1.2) years in boys and 3.9 (1.0) years in girls. During pubertal development, height SDS was unchanged in boys while an apparent increase was observed in girls (**Figure 1**).

Height SDS at T1-T5 for boys and girls is shown in **Table 2**. There was no correlation, in the small subgroup reaching the end of puberty (25 boys and 11 girls), between the duration of treatment in the prepubertal period and total height SDS gain or height SDS gain during the pubertal period.

Maximum height velocity was achieved in T2 (breast development) in girls and in T3 (genital development) in boys (**Figure 2**). For the overall period of puberty, median (range) PPHV was 8.0 (0.3–13.0) cm/year in boys (n=62) and 6.8 (1.3–9.6) cm/year in girls (n=35). PPHV was observed at T2 for 40% of boys and 57% of girls (**Figure 3**) and mean (SD) age at PPHV was 15.3 (1.9) years in boys (n=62) and 13.3 (1.8) years in girls (n=35). In the subgroup of patients reaching T4/T5 at the time of this analysis, mean (SD) PPHV was 8.2 (2.3) cm/year in boys (n=43) and 7.2 (1.5) cm/year in girls (n=23).

TABLE 1 | Patient characteristics of prepubertal[a] patients at the start of rhIGF-1 intake in the Eu-IGFD Registry (baseline) and at last rhIGF-1 intake.

	Boy (n=132)	Girl (n=81)	Total (N=213)
Patient characteristics at the start of rhIGF-1 intake (baseline)			
Previously treated, n (%)[b]	37 (28.0)	19 (23.5)	56 (26.3)
Treatment-naïve, n (%)	95 (72.0)	62 (76.5)	157 (73.7)
Age, years			
Mean (SD)	8.8 (3.8)	8.1 (3.6)	8.6 (3.7)
Median (range)	8.6 (0.4–16.1)	8.2 (1.9–14.8)	8.3 (0.4–16.1)
Height, cm			
n	117	74	191
Mean (SD)	111.3 (20.0)	106.8 (20.8)	109.6 (20.4)
Height SDS			
n	117	74	191
Mean (SD)	-3.7 (1.4)	-4.0 (1.4)	-3.8 (1.4)
BMI SDS			
n	105	68	173
Mean (SD)	-0.7 (1.4)	-0.9 (1.3)	-0.8 (1.4)
Bone age, years			
n	23	16	39
Mean (SD)	7.8 (3.2)	7.1 (3.1)	7.5 (3.2)
Height velocity, cm/y			
n	75	41	116
Mean (SD)	4.6 (1.7)	5.1 (1.8)	4.8 (1.8)
Diagnosis, n (%)[c]			
Severe primary IGF-1 deficiency	116 (87.9)	72 (88.9)	188 (88.3)
Primary IGF-1 deficiency	9 (6.8)	7 (8.6)	16 (7.5)
GH gene deletion with anti-GH antibodies	1 (0.8)	0 (0.0)	1 (0.5)
Small for gestational age	2 (1.5)	2 (2.5)	4 (1.9)
Insulin resistance syndrome	0 (0.0)	1 (1.2)	1 (0.5)
Diabetes	1 (0.8)	0 (0.0)	1 (0.5)
Other	6 (4.5)	2 (2.5)	8 (3.8)
Laron syndrome, n (%)	18 (13.6)	12 (14.8)	30 (14.1)
Patient characteristics at last rhIGF-1 intake[d]			
Age, years			
Mean (SD)	12.9 (4.0)	11.6 (3.6)	12.4 (3.9)
Median (range)	13.0 (2–22)	12.0 (4–18)	12.6 (2–22)
Pubertal stage at last visit while on treatment, n (%)	115	75	190
1	56 (48.7)	38 (50.7)	94 (49.5)
2	14 (12.2)	10 (13.3)	24 (12.6)
3	11 (9.6)	9 (12.0)	20 (10.5)
4	19 (16.5)	12 (16.0)	31 (16.3)
5	15 (13.0)	6 (8.0)	21 (11.1)
Missing data	17	6	23
Height, cm			
n	131	79	210
Mean (SD)	135.1 (22.4)	128.0 (19.7)	132.4 (21.7)
Height SDS			
n	131	79	210
Mean (SD)	-2.9 (1.5)	-3.0 (1.5)	-2.9 (1.5)
BMI SDS			
n	131	79	210
Mean (SD)	-0.1 (1.5)	-0.4 (1.4)	-0.2 (1.5)
Bone age, years			
n	34	16	50
mean (SD)	10.2 (4.4)	10.6 (3.3)	10.3 (4.0)
Height velocity, cm/y			
n	88	51	139
Mean (SD)	5.3 (2.3)	4.7 (2.0)	5.1 (2.2)

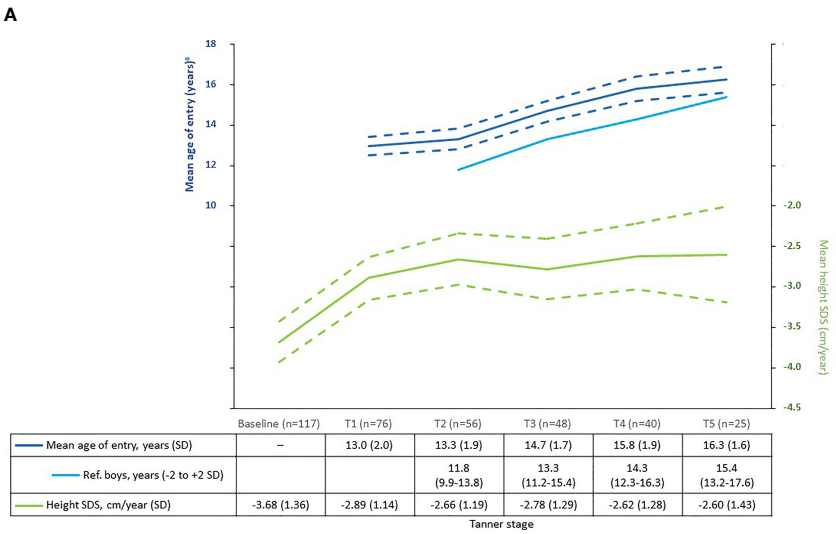
[a]*Prepubertal patients not treated with a gonadotropin-releasing hormone agonist.*

[b]*In prepubertal patients followed until the end of puberty (excluding patients treated with gonadotropin-releasing hormone agonist), 14 were non naïve, including 11 previously treated with rhGH, 2 previously treated with rhIGF-1, and 1 previously treated with both rhGH and rhIGF-1.*

[c]*More than one diagnosis is possible.*

[d]*Or the time of evaluation if treatment with rhIGF-1 was ongoing.*

GH, growth hormone; IGF-1, insulin-like growth factor-1; SD, standard deviation; SDS, standard deviation score.



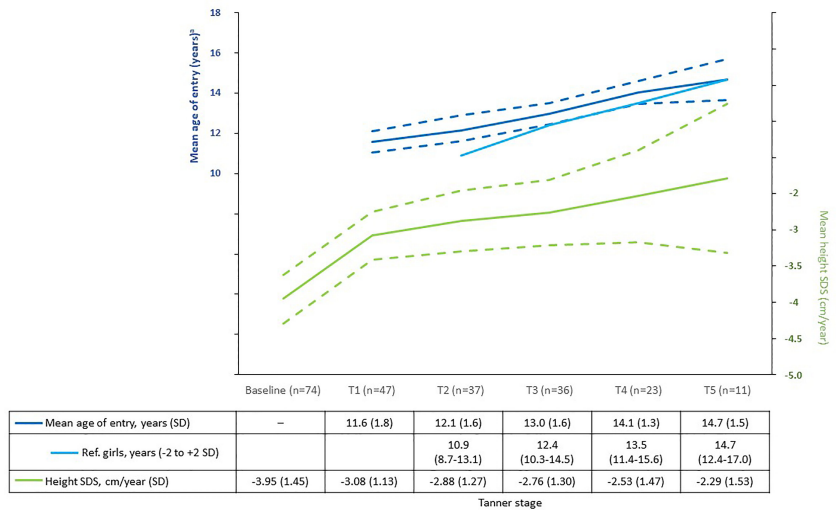


FIGURE 1 | Mean age of entry into Tanner stage* and mean height SDS at each Tanner stage in rhIGF-1-treated children compared with reference population (18). **(A)** Boys. **(B)** Girls. Reference population: healthy Caucasian children from public schools in Denmark between 1991–1993. A total of 826 boys and 1100 girls (aged 6.0 to 19.9 years) were included. Dashed lines show 95% confidence intervals for the Eu-IGFD Registry population. *Except T1 values, which are age at last T1. [a]For children in the Eu-IGFD Registry, this was the mean age at first registration into each Tanner stage. SPIGFD, severe primary insulin-like growth factor-1 deficiency; SD, standard deviation; SDS, standard deviation score.

TABLE 2 | Height SDS at different Tanner stages of the subgroup of children receiving rhIGF-1 who reached the end of puberty during the time period of this analysis[a].

	Boys (n=25)						Girls (n=11)					
	BL	T1	T2	T3	T4	T5	BL	T1	T2	T3	T4	T5
Age in years at Tanner stage, mean (range)	10.9 (5.8 to 15.3)	12.6 (8.0 to 16.0)	13.1 (8.6 to 16.3)	14.2 (11.0 to 16.5)	15.2 (11.9 to 17.4)	16.3 (12.3 to 19.0)	9.1 (6.1 to 11.2)	10.8 (8.8 to 13.0)	11.5 (9.5 to 13.7)	12.4 (10.6 to 15.9)	13.4 (11.0 to 15.4)	14.7 (12.0 to 17.4)
Height SDS, mean (range)	-3.7 (-7.0 to -1.7)	-3.1 (-3.7 to -2.4)	-2.9 (-6.1 to -1.3)	-2.8 (-6.6 to -0.8)	-2.9 (-7.0 to -1.2)	-2.6 (-6.9 to -0.5)	-3.1 (-5.9 to -2.0)	-2.7 (-3.1 to -2.1)	-2.6 (-4.6 to -1.1)	-2.3 (-5.1 to -1.1)	-2.3 (-6.1 to -0.9)	-2.3 (-6.5 to -1.0)

[a]*at latest registration of T1, and at first registration of T2, T3, T4 and T5.*
BL, baseline; SDS, standard deviation score.

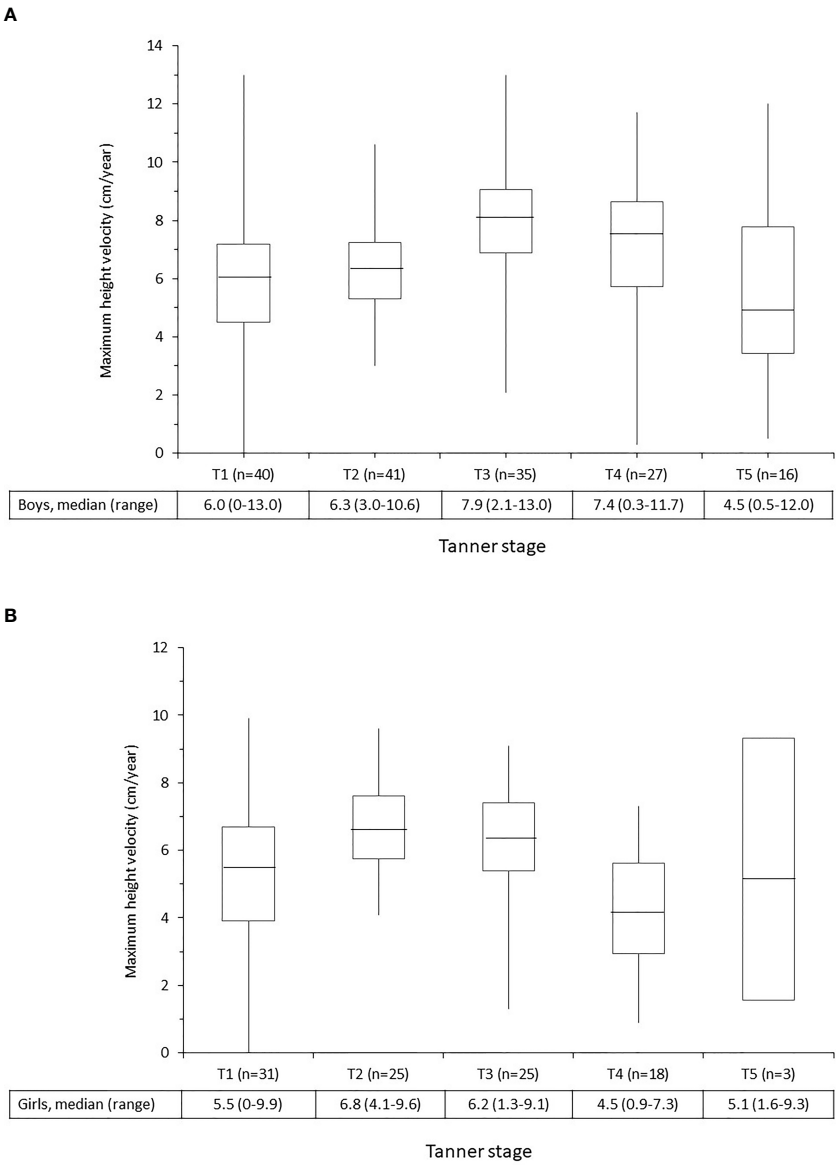


FIGURE 2 | Maximum height velocity at each Tanner stage in children receiving rhIGF-1 for growth failure. **(A)** Boys. **(B)** Girls. The middle box represents the interquartile range; the mid-line represents the median value. The upper/lower whiskers represent the upper and lower quartiles. SPIGFD, severe primary insulin-like growth factor-1 deficiency.

Mean (SD) growth recorded between the last registration of T1 and first registration of T4 was 17.0 (6.4) cm in boys (n=40) and 17.5 (3.8) cm in girls (n=23), and between the last registration of T1 and first registration of T5 was 25.2 (7.2) cm in boys (n=24) and 20.8 (3.9) cm girls (n=11).

Safety

In this population of 213 patients who were prepubertal at the time of initiation of rhIGF-1 treatment, 143 (67.1) had at least one treatment-emergent AE (TEAE; **Table 3**). The three most frequent TEAEs were: hypoglycaemia, 23.9%; lipohypertrophy, 11.7%; and headache, 11.7% (**Table 3**). Targeted TEAEs were reported in 109 patients (51.2%), and 15 patients (7.0%) had 25 serious targeted TEAEs. Twenty-two patients (10.3%) had 39 serious TEAEs that were considered, by the investigator, to be related to treatment. Neoplastic TEAEs were reported in six patients: two cases of melanocytic naevus, and one each of dysplastic naevus, haemangioma of skin, myelodysplastic syndrome and papillary thyroid cancer. Eleven patients (5.2%) withdrew because of TEAEs. Two patients (0.9%) had a fatal TEAE: one patient had myelodysplastic syndrome; and one patient had a complication of a bone marrow transplant. In both patients there were other confounding medical conditions and

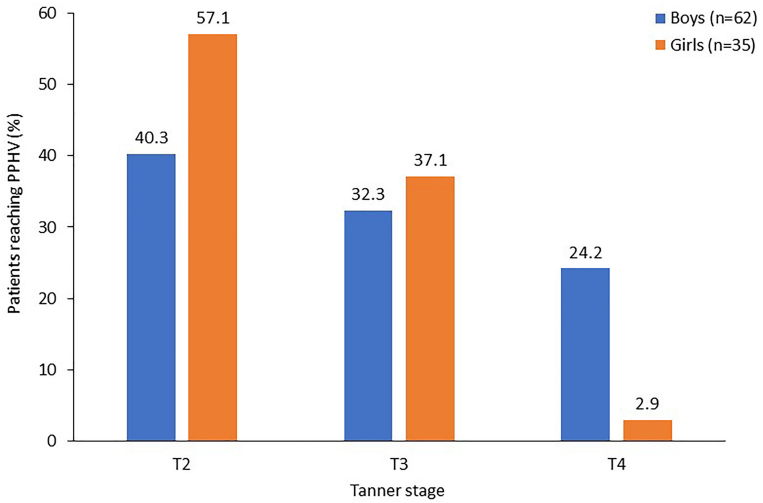


FIGURE 3 | Pubertal stage at pubertal peak height velocity in children receiving rhIGF-1 for SPIGFD with growth failure. Percentage of patients who started puberty with available data on PPHV, and stage at which PPHV occurred. Percentages for each gender do not add up to 100% as Tanner stage 5 has been omitted from this figure. PPHV, pubertal peak height velocity; SPIGFD, severe primary insulin-like growth factor-1 deficiency.

death was considered unrelated to the study drug by the reporting investigators.

DISCUSSION

The results from this analysis of Eu-IGFD Registry population of rhIGF-1-treated patients with SPIGFD and growth failure suggest that puberty is delayed by approximately 1.5 years and that PPHV is delayed in both sexes despite ongoing rhIGF-1 treatment. Treatment with rhIGF-1 may provide improvements in measures of pubertal growth dynamics, including maintenance of (boys) or slight further increase (girls) in height SDS during puberty. The total pubertal height gain in the limited number of patients with SPIGFD reaching T5 during the time period of this analysis was within the expected range for healthy boys and girls, respectively, as was the duration of puberty (last T1 through to T5) (18). The dose of rhIGF-1 may be of importance, and for safety reasons should not exceed 120 μG/kg bid (4), nevertheless in this analysis, not all of the patients received the recommended maximum dose of rhIGF-1. However, the responsiveness to rhIGF-1 is likely to be individual and we have previously failed to identify an rhIGF-1 dose that can predict the first year height response in patients with or without Laron syndrome (12).

Data on age of entry into Tanner stages were compared with a reference population (consisting of 826 and 1100 healthy Caucasian boys and girls, respectively), aged 6.0 to 19.0 years, from public schools in Denmark between 1991 and 1993 (18). These normative data were used because they provide reliable information from a large European population sample. When compared with this reference population, the Eu-IGFD Registry population started puberty approximately 1.5 years later. Laron et al. (19) described reference values for untreated children with SPIGFD, in which the authors noted that puberty was more delayed in boys than in girls: the mean onset of puberty in girls with Laron Syndrome was 10.7 (0.7) years and 15.6 (2.6) years in boys with Laron Syndrome (compared with 12.1 years and 13.3 years, respectively, in the rhIGF-1-treated Eu-IGFD population). Thus rhIGF-1 treatment of patients with SPIGFD does not appear to completely correct the age at which puberty occurs.

The population in the Eu-IGFD Registry reported here reached a maximum height velocity later in life than historical

TABLE 3 | Overview of frequently reported (≥2% of patients) treatment-emergent adverse events in children receiving rhIGF-1 with growth failure[a].

	Number of patients (%)[b] (N = 213)
Any TEAE	143 (67.1)
Serious TEAE	47 (22.1)
Treatment-related TEAE	107 (50.2)
Targeted TEAE	109 (51.2)
Most frequent TEAEs (≥2% of patients)	
Hypoglycaemia	51 (23.9)
Lipohypertrophy	25 (11.7)
Headache	25 (11.7)
Tonsillar hypertrophy	22 (10.3)
Otitis media	17 (8.0)
Insulin-like growth factor increased	12 (5.6)
Deafness	8 (3.8)
Adenoidal hypertrophy	6 (2.8)
Injection site pain	6 (2.8)
Acromegaly[c]	6 (2.8)
Sleep apnoea	5 (2.3)

[a]*Median duration of follow-up from the start of rhIGF-1 treatment, 4.3 years.*
[b]*With at least one event.*
[c]*Acromegalic facial changes*
TEAE, treatment-emergent adverse event; TSH, thyroid stimulating hormone.

healthy controls [15.2 years vs approximately 13.5 years for boys, respectively (20); 13.3 years vs approximately 11.5 years for girls, respectively (20)]. However, untreated children with SPIGFD lack the typical pubertal growth spurt usually seen in children without GH insensitivity (19), and therefore, in the Eu-IGFD Registry population, rhIGF-1 treatment may restore, to a certain extent, the pubertal growth spurt compared with no treatment. Nevertheless, further research is needed to confirm these findings. PPHV was approximately 2 cm/year lower in patients with growth failure included in the Eu-IGFD Registry than in healthy populations (20).

The AE profile reported in this analysis of the Eu-IGFD Registry is generally consistent with previous reports of AEs during long-term treatment with rhIGF-1 (6). rhIGF-1 treatment may increase the risk of benign and malignant neoplasia in patients with SPIGFD (4, 21); therefore, special consideration of these events in this Registry population is important. Although available data do not allow calculations of relative risk, the current analyses included six neoplasm TEAEs (2.8% of the population). In those who receive rhIGF-1 treatment for unapproved uses or at above the recommended doses, risk of neoplasia may be higher. Clinicians should be vigilant for potential malignancy symptoms and if neoplasia develops, rhIGF-1 treatment should be discontinued, and appropriate expert medical care sought. However, the data in this study do not raise any new safety concerns.

While the Eu-IGFD Registry is a robust source of long-term data in a large Europe-wide population, an updated analysis of the data would provide a larger dataset for analysis of near adult height and pubertal growth characteristics. Other limitations in these data stem from the non-interventional nature of the Eu-IGFD Registry. For example, the frequency of visits to physicians may have resulted in some stages of puberty being unrecorded. There are also insufficient data on patients who stopped treatment before puberty. As is typical of registries, there is no comparator group and the use of previously published populations (e.g., from Denmark and the UK) may be sub-optimal, but studies of healthy children across the same geographical range as the Eu-IGFD are lacking. Furthermore, it was not possible to establish representative control populations as the ethnicity, country of origin and immigrant status of the study population were not routinely collected in the Registry. Nevertheless, while this represents a drawback of the current analysis, the use of a control group originating from Europe and inclusion of comparator populations large enough to be considered reliable may mitigate these methodological limitations to some extent. In previous analyses of height data from the Eu-IGFD Registry, we focused on children who were prepubertal and naïve to treatment that may affect growth. In the current analysis, most patients (157 of 213) were treatment naïve, but importantly 14 of the 36 children who reached the end of puberty had received prior growth-promoting therapy, including 11 previously treated with rhGH, 2 previously treated with rhIGF-1, and 1 previously treated with both rhGH and rhIGF-1. We do not yet have data regarding the first-year height response in patients previously treated with growth-promoting therapy compared with treatment-naïve patients, but responses may be lower than in treatment-naïve patients. This means there is a potential risk of underestimating the first-year height response in the group of children that reached T5, and were prepubertal at start of rhIGF-1. It is worth noting here that rhIGF-1 is approved for patients with SPIGFD and GH sufficiency, and therefore, is not considered a reasonable alternative to rhGH treatment in GH-sensitive patients. Despite these limitations, these data are the first of their kind, and therefore do add to our knowledge on the impact of rhIGF-1 on pubertal growth dynamics.

The results from this analysis provide further support to the concept that the GH/IGF-1 axis has a crucial role in gonadal function and pubertal development. While there has been a lack of direct evidence showing the benefit of IGF-1 treatment on pubertal development in patients with SPIGFD, indirect evidence has come from studies in patients with GH insensitivity syndrome, which offer a unique human model to study the effects of congenital IGF-1 deficiency. In these patients, pubertal development is delayed and genitalia and gonads are typically small (8, 9). Furthermore, findings from *in vivo* and clinical studies have demonstrated the importance of IGF-1 in supporting testicular function and steroidogenesis (10, 11).

In conclusion, boys and girls treated with rhIGF-1 for SPIGFD with growth failure experienced an increase in height SDS compared with baseline. rhIGF-1-treated patients entered puberty at an older age than children in a previously reported healthy population; and PPHV was achieved later in life and was lower overall than in a previously reported healthy population. Despite an older age at pubertal start, rhIGF-1 treated children with SPIGFD maintain or slightly increase their height SDS during pubertal years. Current knowledge of IGF-1 biology indicates that IGF-1 could play a role in malignancies in all organs and tissues. Physicians should therefore be vigilant of any symptoms of potential malignancy. If benign or malignant neoplasia develops, rhIGF-1 treatment should be discontinued, and appropriate expert medical care sought immediately. Overall, the AEs seen in this analysis were in line with the known safety profile of rhIGF-1. Data from this analysis suggest that, compared with no treatment, rhIGF-1 may provide improvements for children with growth failure due to SPIGFD.

EU-IGFD REGISTRY STUDY GROUP

Austria: G. Hausler, K. Zwiauer; **Belgium**: M.C. Lebrethon, J. de Schepper; **France**: P. Adiceam, C. Braun, B. Cammas (previously M. Colle), H. Carla-Malpuech, C. Cessans, I. Cloix, M. Cogne, R. Coutant, M. de Kardenet, C. Gayet (previously E. Mallet), P. Hassler, M. Houang, A. Lienhardt, A. Linglart, F. M'Bou, M. Nicolino, F. Njuieyon, M. Petrus, M. Pinget, M. Polak, R. Reynaud, P.F. Souchon, M.T. Tauber, K. Wagner, J. Weill; **Germany:** I. Akkurt, S. Al Sawaf, S. Bechtold, M. Bettendorf, D. Bierkamp-Christophersen, J.-G. Blanke, H.-G. Doerr, A. Enniger (previously H. Leichter), M. Frühwald, K. Hartmann, B. Hauffa, E. Hammer, P.-M. Holterhus, A. Hübner, J. Ittner, C. Jourdan, A. Keller, H.S. Kim-Berger, B. Köster (previously

Rosenbaum), J. Krüger (previously Richter-Unruh), C. Land, F. Lorenzen, T. Meissner, K. Mohnike, M. Morlot, H. Müller, C. Ockert, R. Oeverink, T. Rohrer, R. Pankau, C.-J. Partsch, E. Schönau, A. Schuster, K. Schwab, G. Simic-Shleicher, B. Tittel, K. Warnke (previously W. Bonfig), J. Wölfle; **Italy:** S. Cannavò, M. Cappa, V. Cherubini, G. Citro, D. Concolino, M-F. Fainza (previously L. Cavallo), P. Francesco Perri, L. Guazzarotti (previously G. Vincenzo Zuccotti), A. Lampis, S. Longhi, M. Maghnie, R. Minelli (previously S. Bernasconi), L. Perrone, A. Pilotta, A. Sinisi, G. Weber (previously G. Chuimello), S. Zucchini; **Netherlands**: A. Hokken-Koelega, **Poland:** I. Ben-Skowronek, D. Birkholz, A. Bossowski, M. Hilczer, M. Korpal-Szczyrska, J. Smyczynska, L. Szewczyk; **Spain:** J. Argente, C. Bezanilla, M.-F. Borras, A. Carrascosa, R. de Sotto, R. Diaz, A. Feliu-Rovira, C. Fernandez, M. Ferrer, E. Gallego, F. Hermoso, A. Lechuga-Sancho, C. Luzuriaga, J. Martos, M-F. Moreno-Macian, P. Prieto, C. Rodriguez, J. Sanchez Del Pozo, A. Vela; **Sweden:** P. Bang, K. Ekström, N.-Ö. Nilsson; **United Kingdom:** F. Ahmed, L. Denvir, H. Johnstone, T. Mushtaq, L. Patel, C. Peters (previously K. Hussain), R. Ramakrishnan, S. Rose, N. Shaw, H. Storr.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author. Where patient data can be anonymised, Ipsen will share all individual participant data that underlie the results reported in this article with qualified researchers who provide a valid research question. Study documents, such as the study protocol and clinical study report, are not always available. Proposals should be submitted to DataSharing@Ipsen.com and will be assessed by a scientific review board. Data are available beginning 6 months and ending 5 years after publication; after this time, only raw data may be available.

ETHICS STATEMENT

The ongoing Eu-IGFD Registry is being conducted in compliance with independent Ethics Committees/Institutional Review boards (except in the UK, where ethical review is not required for this type of registry), informed consent regulations, the Declaration of Helsinki, the International Conference on Harmonization, and the Good Epidemiological Practice Guidelines. In addition, the Eu-IGFD Registry adheres to all local regulatory requirements including data protection linked to the use of electronic data. Written informed consent was obtained from the parents or legal guardians and the patient (where applicable) before enrolment and data collection.

AUTHOR CONTRIBUTIONS

All authors contributed to the preparation of this manuscript. All authors reviewed and approved the final version of the manuscript. All authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

FUNDING

This study was sponsored by Ipsen.

ACKNOWLEDGMENTS

The authors thank all patients involved in the Registry, as well as their caregivers, care team, investigators and research staff in participating institutions. The authors thank Martin Gilmour, PhD, of ESP Bioscience Ltd, Crowthorne, UK for providing medical writing support, which was sponsored by Ipsen in accordance with Good Publication Practice guidelines.

REFERENCES

1. Bang P, Polak M, Woelfle J, Houchard A, Group EIRS. Effectiveness and Safety of rhIGF-1 Therapy in Children: The European Increlex Growth Forum Database Experience. *Horm Res Paediatr* (2015) 83:345–57. doi: 10.1159/000371798
2. Cohen P. Overview of the IGF-1 System. *Horm Res* (2006) 65(Suppl 1):3–8. doi: 10.1159/000090640
3. Teissier R, Flechtner I, Colmenares A, Lambot-Juhan K, Baujat G, Pauwels C, et al. Characterization and Prevalence of Severe Primary IGF1 Deficiency in a Large Cohort of French Children With Short Stature. *Eur J Endocrinol* (2014) 170(6):847–54. doi: 10.1530/EJE-14-0071
4. *Increlex (Mecasermin) 10mg/Ml Solution for Injection. Summary of Product Characteristics*. (2021). Available at: https://www.medicines.org.uk/emc/product/384/smpc#gref
5. *Increlex (Mecasermin [rDNA Origin] Injection) Prescribing Information*. (2019). Available at: https://www.evicore.com/-/media/files/evicore/clinical-guidelines/solution/specialty-drugs/mecasermin-rdna-origin-increlex-eff-08012019.pdf
6. Fintini D, Brufani C, Cappa M. Profile of Mecasermin for the Long-Term Treatment of Growth Failure in Children and Adolescents With Severe Primary IGF-1 Deficiency. *Ther Clin Risk Manag* (2009) 5(3):553–9. doi: 10.2147/tcrm.s6178
7. Laron Z. Insulin-Like Growth Factor 1 (IGF-1): A Growth Hormone. *Mol Pathol* (2001) 54(5):311–6. doi: 10.1136/mp.54.5.311
8. Laron Z. Natural History of the Classical Form of Primary Growth Hormone (GH) Resistance (Laron Syndrome). *J Pediatr Endocrinol Metab* (1999) 12(Suppl 1):231–49.
9. Laron Z. Development and Biological Function of the Female Gonads and Genitalia in IGF-I Deficiency – Laron Syndrome as a Model. *Pediatr Endocrinol Rev* (2006) 3:188–91.
10. Colon E, Svechnikov KV, Carlsson-Skwirut C, Bang P, Soder O. Stimulation of Steroidogenesis in Immature Rat Leydig Cells Evoked by Interleukin-1alpha is Potentiated by Growth Hormone and Insulin-Like Growth Factors. *Endocrinology* (2005) 146(1):221–30. doi: 10.1210/en.2004-0485
11. Ekstrom K, Carlsson-Skwirut C, Ritzen EM, Bang P. Insulin-Like Growth Factor-I and Insulin-Like Growth Factor Binding Protein-3 Cotreatment Versus Insulin-Like Growth Factor-I Alone in Two Brothers With Growth Hormone Insensitivity Syndrome: Effects on Insulin Sensitivity, Body Composition and Linear Growth. *Horm Res Paediatr* (2011) 76(5):355–66. doi: 10.1159/000330410
12. Bang P, Woelfle J, Perrot V, Sert C, Polak M. Effectiveness and Safety of rhIGF-1 Therapy in Patients With or Without Laron Syndrome. *Eur J Endocrinol* (2021) 184(2):267–76. doi: 10.1530/EJE-20-0325
13. Chernausek SD, Backeljauw PF, Frane J, Kuntze J, Underwood LE, Group GHISC. Long-Term Treatment With Recombinant Insulin-Like Growth Factor (IGF)-I in Children With Severe IGF-I Deficiency Due to Growth

Hormone Insensitivity. *J Clin Endocrinol Metab* (2007) 92(3):902–10. doi: 10.1210/jc.2006-1610
14. Ranke MB, Savage MO, Chatelain PG, Preece MA, Rosenfeld RG, Blum WF, et al. Insulin-Like Growth Factor I Improves Height in Growth Hormone Insensitivity: Two Years' Results. *Horm Res* (1995) 44(6):253–64. doi: 10.1159/000184637
15. Midyett LK, Rogol AD, Van Meter QL, Frane J, Bright GM, Group MSS. Recombinant Insulin-Like Growth Factor (IGF)-I Treatment in Short Children With Low IGF-I Levels: First-Year Results From a Randomized Clinical Trial. *J Clin Endocrinol Metab* (2010) 95(2):611–9. doi: 10.1210/jc.2009-0570
16. Kelly A, Winer KK, Kalkwarf H, Oberfield SE, Lappe J, Gilsanz V, et al. Age-Based Reference Ranges for Annual Height Velocity in US Children. *J Clin Endocrinol Metab* (2014) 99(6):2104–12. doi: 10.1210/jc.2013-4455
17. Cole TJ, Pan H, Butler GE. A Mixed Effects Model to Estimate Timing and Intensity of Pubertal Growth From Height and Secondary Sexual Characteristics. *Ann Hum Biol* (2014) 41(1):76–83. doi: 10.3109/03014460.2013.856472
18. Juul A, Teilmann G, Scheike T, Hertel NT, Holm K, Laursen EM, et al. Pubertal Development in Danish Children: Comparison of Recent European and US Data. *Int J Androl* (2006) 29(1):247–55. discussion 86–90. doi: 10.1111/j.1365-2605.2005.00556.x
19. Laron Z, Lilos P, Klinger B. Growth Curves for Laron Syndrome. *Arch Dis Child* (1993) 68:768–70. doi: 10.1136/adc.68.6.768
20. Tanner JM, Whitehouse RM, Takaishi M. Standards From Birth to Maturity for Height, Weight, Height Velocity, and Weight Velocity: British Children, 1965. *Arch Dis Child* (1966) 41(454-71):613–35. doi: 10.1136/adc.41.220.613
21. Brahmkhatri VP, Prasanna C, Atreya HS. Insulin-Like Growth Factor System in Cancer: Novel Targeted Therapies. *BioMed Res Int* (2015) 2015:538019. doi: 10.1155/2015/538019

Conflict of Interest: PB has attended advisory boards and received board of directors fees from Ipsen and Lilly, and has received consulting fees from Ipsen, Sandoz, Pfizer, Lilly and Versatis. JW has attended advisory boards and received board of directors fees from Ipsen and Novo Nordisk, and has received corporate-sponsored research fees from Pfizer and Ipsen, as well as speaker fees from Merck-Serono, Hexal, Pfizer and Novo Nordisk. MP has attended advisory boards and received board of directors fees from Ipsen (Increlex Registry), Novo Nordisk (Global Norditropin Advisory board), Pfizer France, and has received corporate-sponsored research fees from Ipsen, Novo Nordisk, Pfizer, Sandoz, Merck and Sanofi, as well as speaker fees from Novo Nordisk and Ipsen. VP and CS are both employees of Ipsen.

The authors declare that this study received funding from Ipsen. The funder contributed to the study design, data collection and analysis, decision to publish, and funded editorial support for preparation of the manuscript.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 28 February 2022
doi: 10.3389/fped.2022.839278

Patients' Perception of the Use of the EasyPod™ Growth Hormone Injector Device and Impact on Injection Adherence: A Multi-Center Regional Study

Asma Deeb[1], Saif Al Yaarubi[2], Bassam Bin Abbas[3], Jamal Al Jubeh[4], Deepti Chaturvedi[5], Noura Al Hassani[6], Angham Mutair[7], Neamat Al Masri[1], Yazan Al Sanad[1]*, Azza Al Shidhani[2], Noha Samir Mahmoud[8], Abdullah Alherbish[8] and Martin O. Savage[9]*

[1] Sheikh Shahbout Medical City & Khalifa University, Abu Dhabi, United Arab Emirates, [2] College of Medicine, Sultan Qaboos University Hospital, Muscat, Oman, [3] King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, [4] Department of Pediatrics, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates, [5] Department of Pediatrics, Burjeel Hospital, Abu Dhabi, United Arab Emirates, [6] Division of Endocrine and Diabetes, Department of Pediatrics, Tawam Hospital and Faculty of Medicine and Health Science, UAE University, Al Ain, United Arab Emirates, [7] Pediatric Endocrine Division, Department of Pediatrics, King Abdulaziz Medical City, King Abdullah Specialist Children Hospital, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia, [8] Alhabib Medical Group, Riyadh, Saudi Arabia, [9] Centre for Endocrinology, Barts and the London School of Medicine & Dentistry, William Harvey Research Institute, Queen Mary University of London, London, United Kingdom

OPEN ACCESS

Edited by:
Sandro Loche,
Ospedale Microcitemico, Italy

Reviewed by:
Giorgio Radetti,
Ospedale di Bolzano, Italy
Chiara Guzzetti,
Ospedale Microcitemico, Italy

***Correspondence:**
Asma Deeb
adeeb@seha.ae
Yazan Al Sanad
o-yalsanad@seha.ae

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: 19 December 2021
Accepted: 31 January 2022
Published: 28 February 2022

Citation:
Deeb A, Al Yaarubi S, Abbas BB, Al Jubeh J, Chaturvedi D, Al Hassani N, Mutair A, Al Masri N, Al Sanad Y, Al Shidhani A, Mahmoud NS, Alherbish A and Savage MO (2022) Patients' Perception of the Use of the EasyPod™ Growth Hormone Injector Device and Impact on Injection Adherence: A Multi-Center Regional Study. Front. Pediatr. 10:839278. doi: 10.3389/fped.2022.839278

Objective: This study aimed to assess patient perceptions of the use of the EasyPod™ growth hormone delivery device and its association with compliance.

Methods: This cross-sectional, multicenter study was conducted in six centers from three countries (United Arab Emirates, Oman, and Saudi Arabia,) between March 2020 and June 2020. Children and adolescents aged 3–18 years, diagnosed with growth disorders and receiving rhGH through the EasyPod™ device were enrolled. Patients and caregivers were given a pre-set questionnaire that evaluated patient satisfaction, preference for technical and personalized features, and device drawbacks. The results were analyzed using independent measures of analysis of variance to evaluate the association of higher satisfaction with device features and better compliance.

Results: A total of 186 patients were enrolled in the study. Of these, 45.7% had GH deficiency. The mean age (±SD) of patients was 11.8 (±2.76) years; 117 (62.90%) were males. Average compliance was 87%. One hundred patients (53.76%) had injection compliance of ≥90%. Amongst these patients, 74%, 68%, and 77% top-scored (5/5) the technical features of hidden needle, skin sensor, and pre-set dosing, respectively, compared to top scores by 39%, 34%, and 51% patients in the <90% compliance group (p-value <0.05). Similarly, a statistically significant difference was observed between the groups (p-value <0.05) in the perception of the usefulness of the tracking features such as display of history of injected doses (78% vs. 47.7%), a reminder for medicine remaining (46% vs. 23.3%) and battery power indicator (48% vs. 20.9%). Personal screen messages were associated with higher compliance while the requirement to keep the

device in the fridge was reported as the most inconvenient feature by 56% of patients in the higher compliance group as against 39.5% in the lower compliance group (p-value <0.05). There was no statistically significant difference in the intensity of pain reported in the two compliance groups.

Conclusion: Our study showed that there is a statistically significant association between better perception of device features and higher compliance.

Keywords: EasyPod™, growth hormone deficiency, recombinant human growth hormone, injector device features, compliance

INTRODUCTION

The availability of recombinant human growth hormone (rhGH) has made GH treatment for short stature widely available (1). Growth hormone treatment has a wide list of indications, some of which have received approval from the Food and Drug Administration or European Medicines Agency. These include GH deficiency (GHD), Turner syndrome, short stature related to a birth size small for gestational age (SGA), idiopathic short stature, and growth failure in pre-pubertal children due to chronic renal insufficiency (CRF) (2–4).

GH therapy has been demonstrated to improve short-term growth and adult height in approved indications (5). However, considerable variability in response has been noted depending on the age at the start of therapy, GH dose, genetic conditions, concomitant illness, and compliance (3, 6). As with any chronic long-term treatment, rhGH treatment is burdened with suboptimal adherence, especially in a pediatric population (7). Factors affecting adherence to GH therapy include the patients' preference for the GH delivery device, its simplicity, and convenience, as well as appropriate education and technical training (8). Daily subcutaneous GH injections can create a significant treatment load, negatively influencing adherence to therapy (2). Adherence to the recommended treatment regimen is important for successful outcomes with rhGH therapy to ensure that patients reach their target height (9). Low adherence is also associated with less favorable clinical outcomes and increased healthcare costs (10).

Several strategies have been proposed to improve adherence. These include improving device simplicity, convenience, and education and training of patients and parents (2). A recent survey of patients, parents, physicians and nurses with experience in the administration of rhGH suggested that reliability, ease of use, lack of pain during injection, safety in use and storage, and a minimum number of steps before injection preparation, were all important factors (10). In addition, a good tracking system to objectively monitor treatment adherence was considered extremely important by the treating physicians (10). Precise information on treatment adherence allows the clinician to exclude poor adherence as a possible reason for sub-optimal growth response, driving further treatment adjustment (2). Multiple long-acting GH (LAGH) preparations are also currently being developed in an attempt to decrease GH injection frequency from daily to weekly, biweekly, or monthly, thereby attempting to improve adherence (11).

EasyPod™ is an electronic auto-injector device that is equipped for adherence monitoring. It has several features including pre-set dosing, adjustable injection settings, and monitoring of adherence using an injection log that records injection history, which can be accessed by patients and clinicians to monitor adherence. The device is equipped with other functions such as screen reminders for battery life, medication cartridge filling, and the number of medication doses left to encourage better compliance. In addition, the device has specific features to encourage children to use it, including protective skin covers, colorful device screen outliners, customized screen messages, and screen photographs of the patients' choice. These features are intended to create a bond between children and their devices and improve compliance.

The present study was carried out to assess patient satisfaction with the technical and tracking features of the EasyPod™ delivery device and its association with compliance. We also aimed to explore the most preferred personal features of the device, patients' scoring of pain severity, and drawbacks of the device. This users' feedback will enable the application of various improvement strategies on the device, encouraging better utilization with the ultimate aim of improving treatment adherence.

MATERIALS AND METHODS

Structure of the Study

This was a questionnaire-based, multicenter, survey study conducted from the 1st of March to the 30th of June 2020. The initial study was planned to be carried out at eight centers from three countries (United Arab Emirates, Oman, and Saudi Arabia), but due to the lack of compliance data from two centers, the final data analysis was carried out with data from six centers. A total of 186 children and adolescents diagnosed with growth disorders in the age group of 3–18 years and receiving rGH (Saizen®, Merck Serono International SA, Geneva, Switzerland) through the EasyPod™ (Merck Serono International SA, Geneva, Switzerland) device were enrolled in the survey. All enrolled subjects were on GH treatment for a variable period, with a mean (±SD) duration of 3.74 (±2.9) years and were using the EasyPod™ device only. Participants above 12 years mostly self-injected, while younger children were helped by their parents/carers to inject. Informed consent was obtained from the participants and/or their parents. The patients' compliance to injectable therapy was reviewed by downloading

the data recorded in the device and is taken as a percentage over the latest 3–6 months period of use.

The aim of the study and details of the questionnaire were explained to the participants by the study team consisting of pediatric endocrinologists and endocrine nurses. The study was approved by local institutional review boards.

Scoring Systems Within the Questionnaire

The questionnaire was designed by co-authors who formed a focus group to assess users' satisfaction and perception of use. It was validated for use by multiple trials in the clinic with staff and users before commencing the study. It included questions on five main areas related to the device (**Appendix**).

1. Patient satisfaction with the technical features (Q1)
2. Patients' views on tracking features and compliance support (Q2-3)
3. Patients' preferred personalized feature (Q4)
4. Patient scoring of pain on device use (Q5)
5. Experience on device drawbacks (Q6)

The Question on satisfaction with technical features (Q1) of the device was scored on a 5-point scale, with 1 being un-useful, 2 less useful, 3 neutral, 4 useful and 5 being very useful. Tracking features were assessed in 2 questions. Question 2 was scored on a 5 points scale (as above) and Question 3 was designed to have an answer of either "Yes," "No," or "Neutral." Inquiry on preferred personalized device features (Q4) was assessed through a multiple-choice question in which a list of personalized features was given to choose from. Pain intensity was scored on a 5- point scale, with 1 being completely painless, 2 minimal, 3 mild pain, 4 moderate pain and 5 being very painful (Q5). Views on device drawbacks were enquired about in a multiple-choice question which users scored for the most appropriate answer (Q6).

Endpoints of the Survey

The primary endpoint of the survey was to assess patient satisfaction with the technical and tracking features of the EasyPod™ delivery device and its association with compliance. The secondary endpoint was to explore patients' preferred personalized device features, pain experience, and device drawbacks. Device compliance of 85% is considered to be satisfactory as per the literature (9, 12). However, considering the overall high compliance in our cohort, we considered compliance of 90% or more as the cut-off level to perform a segmental analysis on this proportion of patients.

Statistics and Data Analysis

Standard descriptive statistics and demographic and primary data analysis was carried out in 186 patients. The proportion analysis of patients who rated the automated dose delivery and tracking features as being "very useful" and helpful in tracking and increasing compliance was summarized using frequency count. Mean (± SD) values were calculated for the baseline characteristics of patients' ages and duration of treatment with GH. The data was divided into two subgroups based on compliance (<90% compliance and ≥90% compliance). For ordinal data obtained from Q1, Q2, and Q5, the Kruskal-Wallis H test (sometimes also called the "one-way ANOVA on ranks") was used to determine if there are statistically significant differences between the two groups (compliance <90% vs. ≥90%) to an independent variable on a continuous dependent variable. For categorical data (Q3, Q4, Q6), univariate logistic regression analysis was used for statistically significant differences between two or more groups (compliance <90% vs. ≥90%).

RESULTS

Subjects and Diagnoses

A total of 186 subjects were enrolled from six centers in three countries (Center 1: 32, Center 2: 12, Center 3: 17, Center 4: 65, Center 5: 40, Center 6: 20). The mean age ± SD of the enrolled patients was 11.8 ± 2.76 years. There were 117 males and 65 females in the study. Gender information was missing for four patients. The mean ± SD duration of treatment with growth hormone was 3.74 ± 2.9 years. The mean percentage of compliance recorded was 87% (range: 50–100%). A hundred patients (53.76%) had injection compliance of ≥90%; mean compliance in this group was 95% (range: 90–100%). The average duration of use of the device in these patients was 3.49 ± 2.98 years compared to 3.56 ± 2.33 in the 86 patients with <90% compliance (mean compliance of 79%; range: 50–89%). Twenty-nine patients had 100% compliance and the average duration of use in these patients was 2.57 ± 2.76 years. The majority of patients were diagnosed with GH deficiency (48.9%) of which 6 patients (6.6%) had panhypopituitarism. Other diagnoses were idiopathic short stature (21.5%), small for gestational age (15.6%), chronic renal failure (3.2%), and Turner's syndrome (3.2%). Other conditions included were Noonan syndrome (2.7%), skeletal dysplasia (2.2%), Fanconi Bickel syndrome (1.1%), rheumatoid disease (0.5%), and osteogenesis imperfecta (0.5%). Data on diagnosis was missing for one patient (**Table 1**).

Scoring of Automated Dose Delivery Features of the Device

The data set for the primary endpoint analysis comprised of 186 patients. The technical features of the device were scored as follows: 74% of patients in the higher compliance group of ≥90% reported the hidden needle feature to be "very useful" compared to 45.3% in the lower compliance group. Similarly, the skin sensor and pre-set dosing facility were scored as "very useful" by 68 and 77% of patients, respectively, in the higher compliance group as compared to 39.5 and 59.3% in the lower compliance group (**Table 2**). A Kruskal-Wallis H test showed that there was a statistically significant difference in perception regarding the usefulness of the hidden needle feature between the two compliance groups (<90% vs. ≥ 90%, [χ^2 (1) = 18.943, $p <$ 0.001]), with a mean rank of 77.31 in the <90% compliant group and 107.42 in the ≥90% compliant group. Similarly, patients in the ≥90% compliance group were statistically significantly more satisfied with the skin sensor feature [mean rank of 78.80 in the <90% compliance group and 106.14 in the ≥90% compliance group; χ^2 (1) = 14.756, $p <$ 0.001], and the pre-set dosing feature [mean rank of 84.56 in the <90% compliance group and 101.18

TABLE 1 | Baseline demographic profile ($N = 186$).

Mean (±SD) age	11.8 (±2.76)
Sex ratio (M:F)[a]	117:65
GH indication[b] *N* (%)	
GH deficiency *N* (%)	91 (48.9)
Panhypopituitarism	6 (6.6%)[c]
Idiopathic short stature *N* (%)	40 (21.5)
Small for gestational age *N* (%)	29 (15.6)
Chronic renal failure *N* (%)	6 (3.2)
Turners syndrome *N* (%)	6 (3.2)
Noonan syndrome *N* (%)	5 (2.7)
Skeletal dysplasia *N* (%)	4 (2.2)
Fanconi Bickel syndrome *N* (%)	2 (1.1)
Rheumatoid disease *N* (%)	1 (0.5)
Osteogenesis imperfect *N* (%)	1 (0.5)
Total	185
Duration of GH indication in years [Mean (±SD)]	3.74 (±2.9)
Average compliance (%)	87%
Mean compliance (<90%) (Range)	79% (50–89%)
Mean compliance (≥90%) (Range)	95% (90–100%)

[a] *Gender information is missing for four patients.*
[b] *Indication data is missing for one patient.*
[c] *Proportion of patients with GH deficiency.*

in the ≥ 90% compliance group; χ^2 (1) = 6.616, $p = 0.010$] compared to patients in the <90% compliance group.

Tracking Features

Among the tracking features, a statistically significant higher proportion of patients (78%) in the ≥90% compliance group reported the feature of the history of injected and missed doses to be "very useful" as compared to 47.7% in the <90% compliance group (<90% vs. ≥90%, [χ^2 (1) = 23.266, $p < 0.001$]) (**Table 2**), with a mean rank of 75.92 in the <90% compliance group and 108.62 in the ≥90% compliance group.

The feature of medicine left in cartridge reminder and the battery power indicator was rated to be "very useful" by 46 and 48%, respectively, by patients in the higher compliance group (**Table 2**). This was significantly higher than the patients in the lower compliance group (23.3 and 20.9%) ($p < 0.001$). A Kruskal-Wallis H test showed that there was a statistically significant difference in perception regarding the cartridge change notification feature between the different compliance groups [<90% vs. ≥90%; χ^2 (1) = 17.908, $p < 0.001$], with a mean rank of 76.18 in the <90% compliance group and 108.40 in the ≥90% compliance group. Similarly, a Kruskal-Wallis H test showed that there was a statistically significant difference in perception regarding the battery power left notification feature between the different compliance groups [<90% vs. ≥90%; χ^2 (1) = 20.375, $p < 0.001$], with a mean rank of 74.96 in the <90% compliance group and 109.44 in the ≥90% compliance group.

Among highly compliant patients, 80% found the tracking features of the device to be useful in tracking missed doses and in encouraging their child to be more compliant. However, a Chi-square test showed that this number was not statistically significantly higher [χ^2 (1) = 1.183, $p = 0.277$] than that in the lower compliance group (<90%).

Personalized Features

A Chi-square test was not statistically significant between compliance groups in choice of personalized device features such as colorful covers [χ^2 (1) = 1.151, $p = 0.562$], device skins [χ^2 (1) = 0.058, $p = 0.810$] and welcome picture [χ^2 (1) = 0.786, $p = 0.375$]. However, the personal screen message feature was significantly [χ^2 (1) = 4.212, $p = 0.040$] associated with a higher (≥90%) compliance status (**Table 3**).

Pain on Injection

In the higher compliance group, 48% of patients reported no pain experience on using the device (score of 1/5) and none reported a score of 5/5 (indicative of severe pain). Further, 49% of patients reported minimal to mild pain (scores of 2/5 and 3/5) in this group. However, a Kruskal-Wallis H test showed that there was no statistically significant difference in pain scores between the two compliance groups [<90% vs. ≥90%; χ^2 (1) = 1.359, $p = 0.244$], with a mean rank of 97.10 for compliance <90% and 88.55 for compliance ≥90% (**Table 4**).

Most Inconvenient Feature

The requirement of keeping the device in the fridge was reported as the most inconvenient feature in the higher compliance group by 56% of patients as compared to 39.5% patients in the lower compliance group (p-value <0.05). However, there was no statistically significant association between choice of others features such as special batteries [χ^2 (1) = 3.395, $p = 0.183$], special needles [χ^2 (1) = 3.145, $p = 0.208$] and heavy device [χ^2 (1) = 4.555, $p = 0.103$] as being the most inconvenient, and compliance status (**Table 5**).

An analysis to evaluate the association between the scores and age of the patient did not show any statistically significant difference (≤10 years and >10 years).

DISCUSSION

In the present study, patients and their parents or caregivers were surveyed over 4 months to evaluate patients' perception of various functionalities of the device and its impact on compliance. The study demonstrated a high GH treatment compliance in children and adolescents (87%), which is more than the minimum percentage recommended to be considered as optimal adherence to hGH administration (85%) (9, 12). The present study results demonstrated that higher scores of patient satisfaction with the technical and the tracking features of the EasyPod™ delivery device were significantly associated with higher compliance.

Over the years, several attempts have been made to understand patient needs and improve device design. The traditional syringes with needles have been replaced with more innovative user-friendly devices that include injection pens, self-injection pens, needle-free devices, and electronic devices with the potential to improve adherence. Adherence to treatment plays a vital role in

TABLE 2 | Comparison between compliance groups for the usefulness of automated dose delivery and tracking features ($N = 186$).

		Compliance (<90%)* ($n = 86$)	Compliance (≥90%)* ($n = 100$)	Total* ($n = 186$)	χ^2 test statistics	*P*-value
Automated dose delivery features						
Hidden needle that auto-injects the medicine	Un-useful	0 (0.0)	0 (0.0)	0 (0.0)	18.943	<0.001
	Less useful	9 (10.5)	1 (1.0)	10 (5.4)		
	Neutral	16 (18.6)	7 (7.0)	23 (12.4)		
	Useful	22 (25.6)	18 (18.0)	40 (21.5)		
	Very Useful	39 (45.3)	74 (74.0)	113 (60.8)		
Skin sensor that helps with injection technique	Un-useful	0 (0.0)	0 (0.0)	0 (0.0)	14.756	<0.001
	Less useful	4 (4.7)	0(0.0)	4 (2.2)		
	Neutral	15 (17.4)	11 (11.0)	26 (14.0)		
	Useful	33 (38.4)	21 (21.0)	54 (29.0)		
	Very Useful	34 (39.5)	68 (68.0)	102 (54.8)		
Preset dosing so no daily dialing is required	Un-useful	0 (0.0)	1 (1.0)	1 (0.50)	6.616	0.010
	Less useful	0 (0.0)	0 (0.0)	0 (0.0)		
	Neutral	15 (17.4)	8 (8.0)	23 (12.4)		
	Useful	20 (23.3)	14 (14.0)	34 (18.3)		
	Very Useful	51 (59.3)	77 (77.0)	128 (68.8)		
Tracking features						
History of injected and missed doses	Un-useful	14 (16.3)	2 (2.0)	16 (8.6)	23.266	<0.001
	Less useful	4 (4.7)	0 (0)	4 (2.2)		
	Neutral	14 (16.3)	6 (6.0)	20 (10.8)		
	Useful	13 (15.1)	14 (14.0)	27 (14.5)		
	Very Useful	41 (47.7)	78 (78.0)	119 (64.0)		
Amount of medicine left in cartridge reminding patients of time to change cartridge	Un-useful	7 (8.1)	2 (2.0)	9 (4.8)	17.908	<0.001
	Less useful	15 (17.4)	5 (5.0)	20 (10.8)		
	Neutral	24 (27.9)	19 (19.0)	43 (23.1)		
	Useful	20 (23.3)	28 (28.0)	48 (25.8)		
	Very Useful	20 (23.3)	46 (46.0)	66 (35.5)		
Battery power left	Un-useful	15 (17.4)	4 (4.0)	19 (10.2)	20.375	<0.001
	Less useful	9 (10.5)	9 (9.0)	18 (9.7)		
	Neutral	26 (30.2)	14 (14.0)	40 (21.5)		
	Useful	18 (20.9)	25 (25.0)	43 (23.1)		
	Very Useful	18 (20.9)	48 (48.0)	66 (35.5)		

**n (%).*

TABLE 3 | Comparison between compliance groups for the preferred personalized device features ($N = 186$).

	Compliance (<90%)* ($n = 86$)	Compliance (≥90%)* ($n = 100$)	Total* ($n = 186$)	χ^2 test statistics	*P*-value
Colorful covers	22 (25.6)	22 (22.0)	44 (23.7)	1.151	0.562
Device skins	16 (18.6)	20 (20.0)	36 (19.4)	0.058	0.810
Welcome picture	20 (23.3)	18 (18.0)	38 (20.4)	0.786	0.375
Personal screen message	44 (51.2)	66 (66.0)	110 (59.1)	4.212	0.040

**n (%).*

the overall clinical outcomes of GH therapy (3). In a large multinational, observational study enrolling children receiving GH treatment through the EasyPod™ device, conducted between 2010 and 2016, it was observed that better adherence to treatment resulted in significant positive growth outcomes (13). Poor compliance resulted in significantly lower growth rates in comparison to patients who missed fewer doses (14).

TABLE 4 | Comparison between compliance groups for the perception of pain with EasyPod™ device ($N = 186$).

		Compliance (<90%)* ($n = 86$)	Compliance (≥90%)* ($n = 100$)	Total ($n = 186$)	χ^2 test statistics	*P*-value
How painful do you find injection with EasyPod	Complete Painless	36 (41.9)	48 (48.0)	84 (45.2)	1.359	0.244
	Minimal Pain	23 (26.7)	30 (30.0)	53 (28.5)		
	Mild Pain	25 (29.1)	19 (19.0)	44 (23.7)		
	Moderate Pain	1 (1.2)	2 (2.0)	3 (1.6)		
	Very Painful	0 (0.0)	0 (0.0)	0 (0.0)		

*n (%).

TABLE 5 | Comparison between compliance groups for the most inconvenient feature of the device.

	Compliance (<90%)* ($n = 86$)	Compliance (≥90%)* ($n = 100$)	Total* ($n = 186$)	χ^2 test statistics	*P*-value
It has to be kept in the fridge	34 (39.5)	56 (56.0)	90 (48.4)	13.971	0.003
Special batteries	20 (23.3)	18 (18.0)	38 (20.4)	3.395	0.183
Special needles	22 (25.6)	27 (27.0)	49 (26.3)	3.145	0.208
Heavy device	39 (45.3)	34 (34.0)	73 (39.2)	4.555	0.103

*n (%).

Studies have suggested that decreased adherence may occur with increasing duration of treatment due to lack of enthusiasm or motivation about adhering to treatment compared with those new users, who may be more diligent (15). In the study by Koledova et al., median adherence rates were high (94%) in the first year of treatment which gradually decreased over follow-up, but a majority of patients maintained ≥80% of adherence over 3 years of treatment (13). In our study too, the average duration of use in patients who had 100% compliance was 2.57 ± 2.76 years, while the entire study population which reported an average duration of use of 3.74 ± 2.9 years showed a lower compliance rate of 87%.

Most common device-related features that impact adherence levels as reported by parents include the product delivery system, its simplicity, convenience, and ease of use, and availability of appropriate training in the administration technique (8, 16). In a study to compare the optimum device for GH administration, a vial combined with an auto-injector or a pen injection system using a cartridge was compared. The study showed that patients preferred auto-injection devices over manual insertion of a needle (17). Dahlgren et al. showed that the auto-injector and skin sensor features of the EasyPod™ device help in increasing the accuracy of auto-injection (18). In an observational 3 month survey with children receiving r-hGH through EasyPod™, 82.5% of participants found the electronic auto-injector easy/very easy to prepare, 92.4% of patients said that the device was easy/very easy to use, 85.0% rated the duration of injection as short/very short and 61.5% reported experiencing no pain when injecting with the electronic auto-injector (19). In our study too, patients were significantly more satisfied with the automated dose delivery features of the hidden needle, skin sensor, and pre-set dosing of the EasyPod™ device and reported a higher compliance rate of ≥90%.

The EasyPod™ device also has improvised display features. The display screen is larger compared to other devices and features high contrast and resolution to be easily read. Studies have shown that the provision of clear instructions affects patient preference for an injection device (20). Our study also demonstrated a high preference for personal screen messages by participants which was associated with higher compliance as well.

In a survey of another autoinjector GH administration device (Sure Pal™), patients or their caregivers rated the dose-memory function as being very helpful/helpful (66.2%). The EasyPod™ device also has an inbuilt electronic adherence monitoring system that provides physicians with personal adherence data. A significantly higher number of patients in the high compliance group of ≥90% found the downloadable and tracking feature of the device to be useful in tracking injected and missed doses. Various other functions such as screen reminders for battery life and medication cartridge filling are also associated with higher compliance.

Stanhope et al., showed that patients experienced less pain with the auto-injector as compared to the pen and also reported less wastage of growth hormone (17). The high patient acceptance and satisfaction of the device in our study are aligned with a favorable safety profile and a high proportion of subjects reporting no pain (45.2%). None of the patients surveyed reported severe pain, and only 3 reported moderate pain as per the pain scale used. We hypothesize that the hidden needle feature of the device could be a factor in reducing the pain as the feature was rated highly by the majority of the subjects. It

is reported that the hidden needle feature is less likely to make the patient anxious and consequently less sensitive toward pain (21). However, there is no statistically significant association in the perception of pain with EasyPod™ and higher compliance.

It can be anticipated that parents well-informed on the diagnosis, and the modalities and problems related to treatment might be more motivated to do the best for their children, to know all possibilities offered by the device. This would once again underline the importance of accurate information given to the parent at the beginning of treatment in order to obtain a better result.

The current study results are in agreement with previous studies using smaller sample sizes and shorter duration and indicate a high level of patient acceptance of the electronic auto-injector for the daily administration of GH. The features of EasyPod™ are considered useful in routine practice and a majority of participants express a desire to continue using the device (22). Patient feedback on drawbacks and pain scores can also provide a basis for improving the technical features and better utilizing the comfort setting for further improving compliance.

A limitation of the study was the design being a cross-sectional open-label survey. The comparison between treatment-naïve and treatment-experienced could not be done. A controlled longitudinal design can be envisaged in the future.

CONCLUSIONS

Patients in the ≥90% compliance group were more satisfied with the automated dose delivery and tracking features of the EasyPod™ device in comparison to those with lesser compliance. Formal education of the device's advanced technical features may further improve satisfaction and ensure injection compliance.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Sheikh Shakhbout Medical City Research Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AD designed the study, collected site data, and liaised between co-authors. All authors contributed equally to the development of the manuscript.

ACKNOWLEDGMENTS

Dr. Rupali Bahri from Medcytes, Dubai provided medical writing services for the development of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.839278/full#supplementary-material

REFERENCES

1. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, et al. Guidelines for growth hormone and insulin-like growth factor-I treatment in children and adolescents: growth hormone deficiency, idiopathic short stature, and primary insulin-like growth factor-I deficiency. *Horm Res Paediatr.* (2016) 86:361–97. doi: 10.1159/000452150
2. Maggio MC, Vergara B, Porcelli P, Corsello G. Improvement of treatment adherence with growth hormone by EasyPod® device: experience of an Italian centre. *Ital J Pediatr.* (2018) 44:113. doi: 10.1186/s13052-018-0548-z
3. Lee PA, Germak J, Gut R, Khutoryansky N, Ross J. Identification of factors associated with good response to growth hormone therapy in children with short stature: results from the ANSWER Program®. *Int J Pediatr Endocrinol.* (2011) 2011:6. doi: 10.1186/1687-9856-2011-6
4. Pfäffle. R. Hormone replacement therapy in children: the use of growth hormone and IGF-I. *R Best Pract Res Clin Endocrinol Metab.* (2015) 29:339–52. doi: 10.1016/j.beem.2015.04.009
5. Collett-Solberg PF, Ambler G, Backeljauw PF, Bidlingmaier M, Biller BMK, Boguszewski MCS, et al. Diagnosis, genetics, and therapy of short stature in children: a Growth Hormone Research Society International Perspective. *Horm Res Paediatr.* (2019) 92:1–14. doi: 10.1159/000502231
6. Bang P, Bjerknes R, Dahlgren J, Dunkel L, Gustafsson J, Juul A, et al. A comparison of different definitions of growth response in short prepubertal children treated with growth hormone. *Horm Res Paediatr.* (2011) 75:335–45. doi: 10.1159/000322878
7. Fisher BG, Acerini CL. Understanding the growth hormone therapy adherence paradigm: a systematic review. *Horm Res Paediatr.* (2013) 79:189–96. doi: 10.1159/000350251
8. Bhosle M, Klingman D, Aagren M, Wisniewski T, Lee WC. Human growth hormone treatment: synthesis of literature on product delivery systems and administration practices. *J Spec Pediatr Nurs.* (2011) 16:50–63. doi: 10.1111/j.1744-6155.2010.00267.x
9. Cutfield WS, Derraik JG, Gunn AJ, Reid K, Delany T, Robinson E, et al. Non-compliance with growth hormone treatment in children is common and impairs linear growth. *PLoS ONE.* (2011) 6:e16223. doi: 10.1371/journal.pone.0016223
10. Loche S, Salerno M, Garofalo P, Cardinale GM, Licenziati MR, Citro G, et al. Adherence in children with growth hormone deficiency treated with r-hGH and the EasyPod™ device. *J Endocrinol Invest.* (2016) 39:1419–424. doi: 10.1007/s40618-016-0510-0
11. Miller BS, Velazquez E, Yuen KCJ. Long-acting growth hormone preparations – current status and future considerations. *J Clin Endocrinol Metab.* (2020) 105:e2121–33. doi: 10.1210/clinem/dgz149
12. Arnao MDR, Sanchez AR, Lopez ID, Fernandez JR, de la Vega JAB, Fernandez DY, et al. Adherence and long-term outcomes of growth hormone therapy with EasyPod™ in pediatric subjects: Spanish ECOS study. *Endocr Connect.* (2019) 8:1240–9. doi: 10.1530/EC-19-0325
13. Koledova E, Tornincasa V, van Dommelen P. Analysis of real-world data on growth hormone therapy adherence using a connected injection device. *BMC Med Inform Decis Mak.* (2020) 20:176. doi: 10.1186/s12911-020-01183-1

14. Desrosiers P, O'Brien F, Blethen S. Patient outcomes in the GH Monitor: the effect of delivery device on compliance and growth. *Pediatr Endocrinol Rev.* (2005) 2(Suppl. 3):327–31.
15. Costello K, Kennedy P, Scanzillo. Recognizing nonadherence in patients with multiple sclerosis and maintaining treatment adherence in the long term. *J Medscape J Med*. (2008) 10:225.
16. Kappelgaard AM, Laursen T. The benefits of growth hormone therapy in patients with Turner syndrome, Noonan syndrome and children born small for gestational age. *Growth Horm IGF Res.* (2011) 21:305–13. doi: 10.1016/j.ghir.2011.09.004
17. Stanhope R, Albanese A, Moyle L, Hamill G. Optimum method for administration of biosynthetic human growth hormone: a randomised crossover trial of an auto injector and a pen injection system. *Arch Dis Child.* (1992) 67:994. doi: 10.1136/adc.67.8.994
18. Dahlgren J, Veimo D, Johansson L, Bech I. Patient acceptance of a novel electronic auto- injector device to administer recombinant human growth hormone: results from an open- label, user survey of everyday use. *Curr Med Res Opin.* (2007) 23:1649–55. doi: 10.1185/030079907X210589
19. Bozzola M, Pagani S, Iughetti L, Maffeis C, Bozzola E, Meazza C. Adherence to growth hormone therapy: a practical approach. *Horm Res Paediatr.* (2014) 81:331–5. doi: 10.1159/000357975
20. Gluckman PD, Cutfield WS. Evaluation of a pen injector system for growth hormone treatment. *Arch Dis Child.* (1991) 66:686–8. doi: 10.1136/adc.66.6.686
21. Dahlgren J. Easypod: a new electronic injection device for growth hormone. *Expert Rev Med Devices.* (2008) 5:297–304. doi: 10.1586/17434440.5.3.297
22. Tauber M, Payen C, Cartault A, Jouret B, Edouard T, Roger D. User trial of Easypod, an electronic autoinjector for growth hormone. *Ann Endocrinol.* (2008) 69:511–6. doi: 10.1016/j.ando.2008.04.003

Conflict of Interest: This publication received funding from Merck Serono Middle East FZ-LTD. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication. This publication is done via an unrestricted medical writing grant by Merck Serono Middle East FZ-LTD, an affiliate of Merck KGaA, Darmstadt, Germany.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 01 March 2022
doi: 10.3389/fendo.2022.795224

Treatment Adherence to Injectable Treatments in Pediatric Growth Hormone Deficiency Compared With Injectable Treatments in Other Chronic Pediatric Conditions: A Systematic Literature Review

Roy Gomez[1], S. Faisal Ahmed[2], Mohamad Maghnie[3,4], Dejun Li[5], Toshiaki Tanaka[6] and Bradley S. Miller[7]*

[1] Global Medical Affairs, Pfizer, Ixelles, Belgium, [2] Developmental Endocrinology Research Group, School of Medicine, University of Glasgow, Glasgow, United Kingdom, [3] Clinica Pediatrica, IRCCS Istituto Giannina Gaslini, Genoa, Italy, [4] Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genoa, Genoa, Italy, [5] Center for Prenatal Diagnosis and Reproductive Medicine, The First Hospital of Jilin University, Changchun, China, [6] Tanaka Growth Clinic, Tokyo, Japan, [7] Pediatric Endocrinology Division, University of Minnesota Masonic Children's Hospital, Minneapolis, MN, United States

OPEN ACCESS

Edited by:
Dennis Michael Styne, University of California, Davis, Davis, United States

Reviewed by:
Domenico Corica, University of Messina, Italy
Hugo Fideleff, University of Buenos Aires, Argentina
Claudio Giacomozzi, Azienda Ospedaliera Carlo Poma, Italy

***Correspondence:**
Roy Gomez
Roy.Gomez@pfizer.com

This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Specialty section:
Received: *14 October 2021*
Accepted: *28 January 2022*
Published: *01 March 2022*

Citation:
Gomez R, Ahmed SF, Maghnie M, Li D, Tanaka T and Miller BS (2022) Treatment Adherence to Injectable Treatments in Pediatric Growth Hormone Deficiency Compared With Injectable Treatments in Other Chronic Pediatric Conditions: A Systematic Literature Review. Front. Endocrinol. 13:795224. doi: 10.3389/fendo.2022.795224

Background: Pediatric patients with growth hormone deficiency (GHD) are currently treated with daily injections of recombinant human growth hormone (rhGH) to promote linear growth and enable attainment of normal adult height. One of the main reasons for suboptimal growth during rhGH therapy is non-adherence to treatment. The objective of this systematic literature review was to examine the recent literature on pediatric adherence to injectable treatments for chronic conditions (focusing on rhGH) to characterize levels of adherence and identify the factors/barriers associated with adherence.

Methods: The Embase and MEDLINE databases (January 2015–October 2020) were searched to identify publications describing studies of pediatric patients (aged ≤17 years) with GHD and other chronic conditions requiring daily or weekly injectable treatments; a similar targeted search of Chinese literature was also performed. Adherence data were extracted from the included studies and summarized. Risk of bias was determined using the Cochrane Risk of Bias tool 2 or the Newcastle-Ottawa Scale.

Results: A total of 23 publications were included, with all publications except for one (multiple sclerosis) focused on pediatric GHD studies: there were two clinical trials, 18 observational studies and three survey studies. Study sample sizes ranged from 30 to 13,553 patients (median: 95 patients). The definition of adherence varied between studies and included mean adherence rate, median adherence rate, and the percentage of patients within pre-specified adherence categories. Of the publications assessing adherence to daily rhGH, 11 studies reported 12-month mean adherence rate (range: 73.3%– 95.3%) and eight studies reported median adherence (range: 91%– 99.2%). The

barriers to treatment adherence identified included self-administration, increased administration frequency, age (adolescence), longer treatment duration, device design, and insufficient family education, awareness, and/or engagement. Recommendations for increasing adherence included using adherence reminder tools, increasing patient engagement/education, and improving injection device design and drug product.

Conclusions: Adherence to rhGH treatment was high (>80%) for many studies, though comparability between studies was limited given the substantial heterogeneity in the way adherence was defined, measured, and reported. To address this heterogeneity, we recommend standardizing how adherence is defined and reported and encourage the use of standardized study designs and outcome measures.

Keywords: adherence, injection, growth hormone, growth hormone deficiency, pediatric, systematic literature review

INTRODUCTION

Growth hormone deficiency (GHD) is characterized by insufficient secretion of human growth hormone (hGH) from the pituitary gland and low serum concentrations of insulin-like growth factor-1 (IGF-1). In children with GHD, growth of skeletal and muscle mass is reduced, resulting in delayed puberty and height below the normal range. Treatment for GHD consists of recombinant hGH (rhGH), which has been shown to promote linear growth in children with GHD, enabling them to achieve normal height in adulthood (1). Currently, most forms of rhGH require administration *via* daily subcutaneous (SC) injections. Despite the efficacy of rhGH and an increased range of injection devices available to patients (1, 2), treatment results can be suboptimal (3–5). The failure of patients with GHD to reach target adult height despite receiving rhGH treatment remains a prevalent outcome (1, 6).

Although the reasons for suboptimal outcomes following rhGH treatment are likely to be multifactorial, it is widely acknowledged that non-adherence to daily injections plays an important role. A study of 217 GHD patients from six pediatric endocrinology clinics in Turkey found that height velocity (HV) and HV standard deviation scores (SDS) in patients with optimal adherence to rhGH therapy were higher than in patients with suboptimal adherence, as were levels of IGF-1, which was correlated with HV and HV SDS (7). Two systematic literature reviews (SLRs) in the last decade examined the paradigm surrounding non-adherence (1, 8). The earlier SLR (1) found that 5–82% of patients miss at least some rhGH doses. Fisher et al. identified the following injection-related factors as being associated with non-adherence: perceived difficulty of injections; lack of choice of injection device; short duration of prescriptions; and discomfort (1). The later SLR by Graham et al. (8) reported that 7–71% of pediatric GHD patients were non-adherent. Of the 22 factors identified as being associated with non-adherence, those related to daily injections included: injection-related pain and discomfort; poor administration technique; forgetting injections; disruption in supply of injections due to short duration of prescriptions; and being away from home (8).

In order to better characterize the levels of adherence and the factors associated with adherence in pediatric patients with GHD, a review of the literature was performed to consider the most recent evidence from the field. To capture insights beyond the existing literature in this field (1, 8), the review also considered evidence from other pediatric therapeutic areas that require regular (daily or weekly) self- or caregiver-administered injectable treatments. These included multiple sclerosis (MS), juvenile idiopathic arthritis (JIA), and inflammatory bowel disease (IBD). Specifically, this review investigates the drivers and barriers to rhGH adherence, as well as recommendations and best practices that can be applied to improve adherence to rhGH.

The objectives of this SLR were to (i) summarize the recent literature on pediatric adherence to injectable treatments for chronic conditions, with a focus on rhGH, and (ii) identify factors associated with pediatric adherence/non-adherence to rhGH and other injectable treatments.

METHODS

This review was guided by the principles of the Interim Guidance from the Cochrane Rapid Reviews Methods Group (9) and guidance from the Centre for Reviews and Dissemination (10). The eligible study populations, interventions, comparators, outcomes, and study types (PICOS) are described in detail in **Table 1** and briefly below.

Eligibility Criteria

Studies evaluating pediatric patients (ages 17 years and younger) with GHD and other chronic conditions (MS, JIA, or IBD) requiring daily or weekly injectable treatments were eligible for inclusion in this review. Studies that included young adults (e.g.,

Abbreviations: ECOS, easypod™ connect observational study; GH, growth hormone; GHD, growth hormone deficiency; hGH, human growth hormone; IBD, inflammatory bowel disease; IGF-1, insulin-like growth factor-1; ISS, idiopathic short stature; JIA, juvenile idiopathic arthritis; MS, multiple sclerosis; NOS, Newcastle–Ottawa Scale; RCT, randomized controlled trials; rhGH, recombinant human growth hormone; SC, subcutaneous; SD, standard deviation; SDS, standard deviation scores; SLR, systematic literature review.

TABLE 1 | Summary of eligibility criteria.

	Included	Excluded
Population	• Children aged <18 years with GHD, an rhGH- indicated condition, or a chronic condition requiring daily or weekly self- or caregiver-administered injectable treatment (MS, JIA, IBD) • Parents or caregivers of pediatric patients treated with regular injections for these conditions	• Studies in adults or where outcomes of pediatric patients are not reported separately from those of adult patients
Intervention	• rhGH or a self- or caregiver-administered injectable drug (SC or IM) indicated for ongoing daily or weekly treatment of chronic conditions (MS, JIA, IBD) in pediatric populations	• Interventions that are not delivered by SC or IM injection (i.e., topical, oral, or infusion) • Interventions that are not identified as SC or IM injection only and that could include other administration routes (e.g., "biologics" if this category includes infused or IV agents) (**Supplementary Table S1**)
Comparator	• Any or none	• Not applicable
Outcomes	• Clearly identifiable/defined standardized measures (validated or non-validated) **AND** reported prevalence of adherence/non- adherence or compliance/non-compliance to injectable drugs, **OR** • Explicitly identifiable and measured (a) barriers to adherence, **OR** (b) characteristics of patients, families/caregivers, providers, or institutions associated with adherence to prescribed treatment, **OR** (c) properties of the treatment, such as administration route or schedule, associated with adherence	• Publications that (a) do not define how adherence was measured, or (b) do not report rate of adherence/non-adherence (e.g., discontinuation or persistence only would be excluded), or (c) do not report barriers/factors affecting adherence • For inclusion, outcomes must be reported for SC or IM injectable drugs separately from infusion/IV, oral, or other routes of administration (**Supplementary Table S1**)
Study design	• Observational studies (prospective or retrospective; including cohort studies, cross- sectional studies, or surveys) • RCTs or non-RCTs (if reporting medication adherence or compliance)	• Studies with non-empirical, theoretical, or narrative discussion of adherence and no quantitative measure of adherence • Publications reporting methods or tool development, unless they report either a quantitative measure of adherence/non-adherence or factors associated with adherence • Other study designs were not eligible (e.g., pre-clinical, case reports/studies reporting patient- level data only, economic studies, pooled data analyses, or meta-analyses) • Systematic reviews published from 2015 onwards were not eligible for inclusion but were hand-searched for additional relevant references
Limits	• Published in peer-reviewed journal from 2015 to 2020 • Studies not published in English will be considered, with data extraction limited to English language elements and numerical data	• Unpublished data and data from conference abstracts

GHD, growth hormone deficiency; IBD, inflammatory bowel disease; IM, intramuscular; IV, intravenous; JIA, juvenile idiopathic arthritis; MS, multiple sclerosis; RCT, randomized contrail trial; rhGH, recombinant growth hormone; SC, subcutaneous.

ages 18–25 years) were eligible if they also included patients younger than 18 years of age, meaning that some patients in the transition period could be included. Studies reporting on self- or caregiver-administered rhGH or an injectable drug, *via* an SC route, for ongoing daily or weekly treatment of chronic conditions (MS, JIA, or IBD) in pediatric populations were eligible for inclusion. Studies with and without comparators were eligible.

Publications were eligible for inclusion if they reported one or more of the following outcomes: (i) clearly defined standardized measures of adherence/non-adherence or compliance/non-compliance AND reported prevalence of adherence/non-adherence or compliance/non-compliance to injectable drugs or (ii) identifiable and measured (a) barriers to adherence OR (b) characteristics of patients, families/caregivers, providers, or institutions associated with adherence/non-adherence to treatment OR (c) properties of the treatment associated with adherence/non-adherence. Observational studies (prospective or retrospective; including cohort studies, cross-sectional studies, or surveys), or randomized controlled trials (RCTs) or non-RCTs (if reporting medication adherence or compliance) were eligible for inclusion. Publications were required to have been published in peer-reviewed journals between 2015 and 2020.

Study Selection

Searches were conducted in the following databases: Embase® *via* Ovid and MEDLINE® *via* Ovid. A detailed search strategy for each database is presented in **Supplementary Methods 1** and **Supplementary Tables S1, S2**. From the records identified in the database searches, one researcher (CR) excluded records that were irrelevant, which were then checked by a second researcher (KS). One researcher (KS) screened the titles and abstracts (Level 1) of the identified publications against the eligibility criteria. A second researcher (CR) screened 20% of the records to ensure agreement of screening decisions. The records identified in Level 1 were then subjected to full-text screening (Level 2) using the same process (initial screen by KS followed by validation of 20% of the records by CR). Screening discrepancies were discussed and resolved.

In addition to the Embase and MEDLINE searches, a targeted search of the Chinese literature was also conducted to identify additional publications on adherence to rhGH therapy in pediatric populations. An author fluent in Chinese (DL) conducted a

literature search for publications in Chinese in the Wan Fang database (http://www.wanfangdata.com.cn/index.html).

Data Extraction and Reporting

One researcher (KS) extracted data from the included studies into a pre-specified data-extraction table (DET) in Microsoft Excel. A second researcher (CR) quality-checked 100% of the extracted data against the original publications for accuracy. For consistency, where available, data were extracted for 12 months or for the latest timepoint available. A list of data points extracted is provided in **Supplementary Methods 2**. For studies reporting mean adherence, the population-weighted mean across studies was calculated. The population weights were the sample size divided by the total number of patients with mean adherence over 12 months in the included studies. The weights were multiplied by the adherence in each study, then summed to obtain the weighted mean.

Risk of Bias

As part of the data-extraction process, one researcher (KS) quality-assessed the RCTs for bias using the Cochrane Risk of Bias tool 2 (RoB2). Observational studies, including cohort studies, surveys, and cross-sectional studies, were quality assessed using the Newcastle–Ottawa Scale (NOS) for observational studies. A second researcher (CR) checked all of the quality assessment ratings; any differences were resolved through discussion. Risk-of-bias assessment was not conducted for studies identified in the targeted search of the Chinese literature.

RESULTS

Study Selection

A total of 1058 references were identified from the database searches; following removal of duplicates, 946 references underwent title/abstract screening, and 893 were excluded (**Figure 1**). Full-text screening was then performed on 55 references (53 from literature databases and two identified from hand search), and a total of 23 publications met the inclusion/exclusion criteria and were subjected to data extraction and quality assessment.

Study Characteristics

The details of each study are provided in **Supplementary Table S3**. Of the 23 studies that met the inclusion criteria, only one study (11) was in an indication (MS) outside of pediatric growth disorders. Study sample sizes ranged from 30 (12) to 13,553 (13) patients, with a median sample size of 95 patients. More than half of the sample population was male in all studies (median 41.5% female) except for the MS study, which was 70% female (11). The mean age of patients among the 17 rhGH studies that reported age ranged from 6 years (for patients receiving daily rhGH) (14) to 12.3 years (15). A total of 6 (26%) studies were from Italy (11, 16–20), five (22%) were from multiple countries (13, 14, 21–23), and three (13%) were from Spain (4, 12, 24). The remaining nine studies were each from a different country (15, 25–32).

Two of the studies were clinical trials. REAL 3 (14) was a Phase 2 randomized, active-controlled, open-label study of short-acting (daily) *vs*. long-acting (weekly, three different dosages) rhGH. SYNERGY (27) was a randomized, open-label study of growth outcomes following 12 months of daily rhGH treatment compared with 6 months' delay followed by 6 months of daily rhGH; adherence for the 12-month treatment arm is reported in this review. Other than the REAL 3 trial (14), which reported adherence to short-acting *vs*. long-acting rhGH, the remaining 21 rhGH adherence studies were of daily GH.

Critical Appraisal

The 23 included studies were subject to critical appraisal/quality assessment using the quality assessment tool appropriate for each

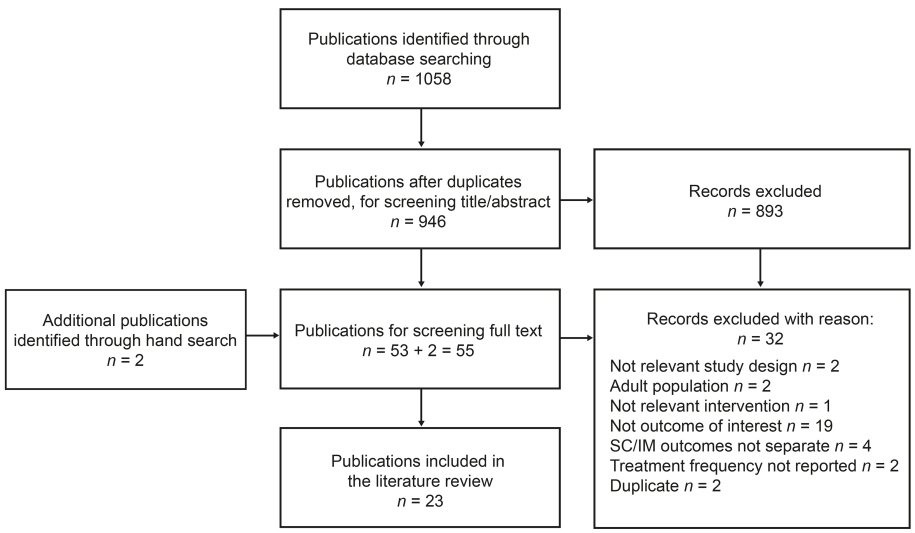


FIGURE 1 | PRISMA diagram of the literature-screening process. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

study type. The reviewers (KS and CR) initially agreed on all ratings except for 4 of the observational cohort studies, which they then discussed until agreement was reached. Both of the RCTs (14, 27) were identified as having a moderate risk of bias using the RoB2 tool (**Supplementary Table S4**). Both RCTs collected self-reported adherence, which could have been subject to bias. Further, the 2018 study by Chung et al. (27) was an open-label study, and the 2020 study by Sävendahl et al. (14) was partially blinded, which could have introduced bias. Study quality for the 18 observational cohort studies was assessed using the NOS (**Supplementary Table S5**). Four studies were rated as good quality, and 14 as fair quality; none of the observational cohort studies had a comparator intervention or control group. Of the 18 studies, 12 did not control for differences among patients in the cohort, which may have influenced results. The quality of the three survey studies (15, 16, 21) was assessed using a modified NOS (**Supplementary Table S6**). One study was of fair quality, and two were of low quality; none of the survey studies reported participation rates or information on non-respondents.

Adherence (Definition and Reporting)

Broadly, adherence in these studies was assessed by several different methods (depending on the study): number of administrations/prescribed doses, as monitored by a medical device [*n* = 12/23 (52.2%)], self-reported number of missed doses for a given time period [*n* = 7/23 (30.4%)], or quantity of pharmacy fills or product supplied/quantity prescribed (*n* = 4/23 [17.4%]). Details of the definition of adherence used in each study are provided in **Supplementary Table S7**. All of the rhGH studies that monitored adherence using a medical device used the easypod™ (Merck) electronic drug-delivery device. The studies also differed in terms of how they reported adherence; some studies reported mean or median adherence rate, whereas others reported the percentage of patients within different pre-specified categories (which varied across studies). Some studies reported adherence using more than one of these measures.

Mean Adherence

Of the 22 rhGH studies, 11 reported the 12-month mean adherence rate to daily rhGH (**Figure 2**). One study compared daily rhGH against weekly rhGH (three different doses) and found that the mean 12-month adherence rate was 91.8% for daily treatment *vs*. 97.5%, 98.6%, and 96.3% for the weekly doses (14); the remaining rhGH publications reported adherence to daily rhGH. The population-weighted mean adherence rate among the 11 studies was 79.3%. Mean adherence rates across the 11 studies ranged from 73.3% (29) to 95.3% (24).

Of the 11 studies, Farfel et al. was different from the others because it measured adherence to rhGH based on the number of months with a pharmacy fill (and not days' supply) (29). Eight of the 11 studies measured adherence using a medical device (all easypod™); the mean adherence rate across these eight studies ranged from 80.8% (in year 0–1, *n* = 95 patients) (22) to 95.3% (*n* = 232 patients) (24). In the single study on MS, the mean 12-month adherence rate (measured using a medical device; **Supplementary Table S7**) to interferon beta-1a among 40 pediatric patients was 67.5% (11).

Median Adherence

A total of 8/22 rhGH studies reported median adherence to daily rhGH (**Figure 3**), and of these, most (6/8) studies used a medical device (easypod) to monitor adherence. Median adherence to daily rhGH was high, ranging from 91% in a single-center retrospective observational study from Germany (31) to 99.2% in an open-label, multicenter RCT (14) (**Figure 3**).

Categorical Measures of Adherence

Many of the studies referred to adherence categories, using terms such as "adherent" or "non-adherent" or "poor," "fair," "good," or "excellent" adherence; details are described in **Supplementary Table S7**. Of the studies reporting adherence as a category, the largest number of studies defined patients as adherent (having "good adherence") to daily treatment if they missed <1 dose per week or were administered >85% of prescribed doses (12, 13, 20, 24, 26, 30, 31). Six studies reported the percentage of patients with adherence >85% or with <1 missed dose per week (**Figure 4**) (4, 12, 13, 16, 30, 31). In a 2015 study of 103 patients that defined adherence as possession of >85% of prescribed doses (pharmacy fills), Lass and Reinehr reported that 51% of patients were considered adherent (31). In the largest

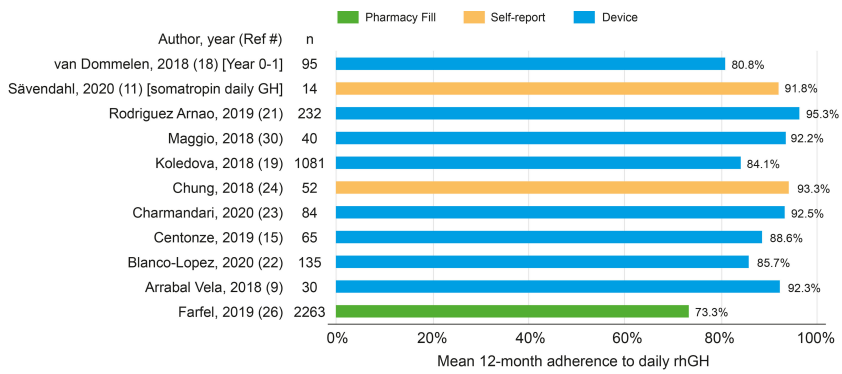


FIGURE 2 | Mean 12-month adherence among 11 rhGH studies. rhGH, recombinant human growth hormone.

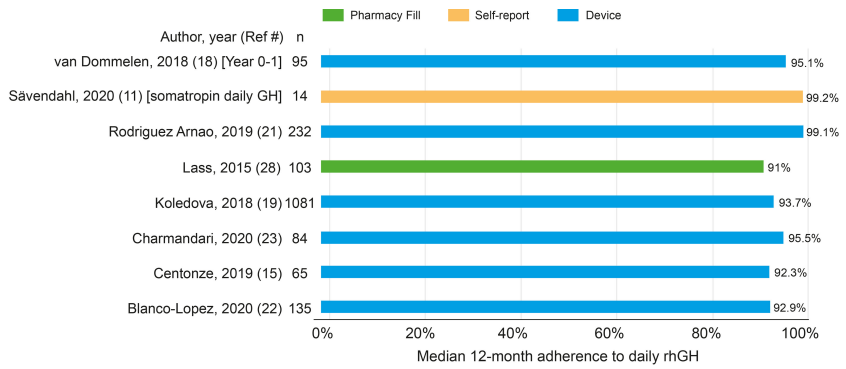


FIGURE 3 | Median 12-month adherence among eight rhGH studies. rhGH, recombinant human growth hormone.

study population included in this review, Koledova et al. reported in 2020 that 77% of 8163 patients with 12 months of data had injected >85% of the prescribed dose of rhGH, as measured by the injection device (13).

A clinical trial by Chung et al. (27) defined non-adherence as receipt of <75% of expected injections based on self-reported diary entries. Based on this definition, adherence (receipt of ≥75% of expected injections) in patients who received treatment for 12 months was 93.27%. Using the same definition, adherence in the control group (patients untreated for the first 6 months and then treated with rhGH for 6 months) was 95.69% (27). Adherence would have been lower if the more commonly used definition, receipt of >85% of expected injections, was used to define adherence.

Two studies used ≥80% to define good adherence (15, 32). The study by Michaelidou et al. (32) was a single-center study from the United Kingdom that measured adherence based on the proportion of days covered, defined as the quantity of jet-injector device-heads delivered, multiplied by the length of time each head should last (1 week), divided by the number of days rhGH treatment was prescribed during the treatment period (**Supplementary Table S7**) (32). According to this definition, 57.5% of patients had sufficient device-heads to be considered adherent.

Easypod Connect Observational Study (ECOS) and Other Easypod Studies

The medical device easypod was the most commonly used method of measuring adherence. Of the 22 included rhGH studies, 11 used easypod to measure adherence to somatropin; these studies (identified by **) are described and adherence reported in **Supplementary Table S7**; most of these studies were sponsored by Merck. ECOS was a Phase 4, open-label study spanning 5 years and 24 countries that measured adherence to rhGH (Saizen, Merck) administered by the easypod. Mean adherence in the primary ECOS (23), which included patients with GHD, small for gestational age, and Turner syndrome from 24 countries, was 84.1%, with a median adherence of 93.7% (23).

In addition to the primary ECOS (23), five different ECOS sub-analyses (all prospective observational studies) met the SLR inclusion criteria. One of these was a 24-country sub-analysis restricted to children with idiopathic isolated GHD (22), wherein the mean (standard deviation [SD]) adherence in the first year

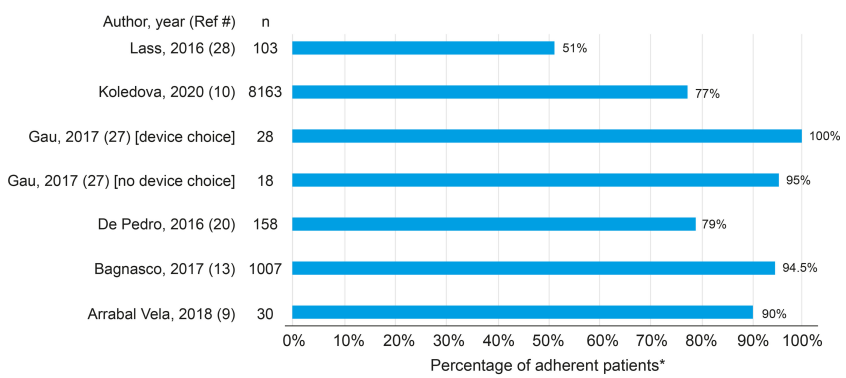


FIGURE 4 | Percentage of patients with adherence > 85%. *Adherence > 85% defined as missed < 1 dose per week, or patients were administered or had in possession > 85% of prescribed doses.

was 80.8% (31.1%) with a median of 95.1%. In the second year, the mean (SD) adherence was 81.5% (23.0%), and the median was 92.9%. The remaining four ECOS were specific to certain countries (Mexico, Greece, Spain, and Italy), with a mean adherence ranging from 85.7% (Mexico) (25) to 95.3% (95% confidence interval: 93.3–97.2) (Spain) (24). Median adherence was above 92% for all four studies and ranged from 92.3% (Italy) (18) to 99.1% (Spain) (24).

In addition to the six ECOS, there were five other studies that used easypod to collect adherence data; three from Italy (17, 19, 20), one from Spain (12), and one large multi-country study of 13,553 patients described above (13). All were retrospective studies except for one by Loche et al. (19), which was a prospective observational study. Of the three studies that reported mean adherence rate, values ranged from 70% ± 13% (time period not reported) (17) to 96% over 12 months (20).

Chinese Literature Survey

The targeted search of the Chinese literature identified two publications, only one of which met the SLR inclusion criteria. A 2020 randomized controlled trial by Li and Liu compared long-acting polyethylene glycol rhGH with short-acting daily rhGH. The investigators reported the number of missed doses out of the total number of prescribed doses for each treatment arm over 26 weeks (33). Adherence was very high in both treatment groups (>99%); however, the difference between adherence in the combined long-acting rhGH (4/1066 missed doses, or 99.62% adherence) and short-acting rhGH (70/7280 missed doses, or 99.04% adherence) was statistically significant (33).

Barriers to Adherence

Several barriers to adherence were identified in the included publications. Older age/adolescence was identified as a barrier in five studies (4, 12, 20, 31, 32). One study by Maggio et al. that looked at different age groups found a lower adherence in patients aged ≤9 years or ≥14 years compared with those aged 10–13 years (20). A longer treatment duration was also associated with a lower adherence, as described in six studies (4, 12, 16, 20, 29, 31). A number of barriers were identified in single studies, and these included: self-administration (*vs.* caregiver-administered) of rhGH (31); lower education level of the patient's mother (4); prescribed seven doses of rhGH compared with six doses administered per week (20); and lack of family education/awareness and engagement (16). Device design could potentially also be a barrier as patients who considered the injection device to be 'not convenient at all' were less likely to be adherent when compared with patients who considered the device 'very convenient' (16). None of these studies reported statistically significant differences in adherence by gender or growth outcomes.

Recommendations for Improving Adherence

The studies identified in this review recommended multiple methods of improving adherence (**Table 2**). Broadly, most of the recommendations were aimed at interactions between patients and the health care team, in addition to the use of reminder tools to improve adherence. Recommendations were also proposed for the injection device and the drug product (**Table 2**). Recommendations for the injection device focused on improving device design in order to reduce pain and needle anxiety and to increase convenience. It was suggested that adherence might also be improved if the drug product did not require cold storage and was able to be injected less frequently than once per day.

DISCUSSION

This SLR was undertaken to provide an overview of pediatric patient adherence to rhGH and other injectable treatments as well as to identify some of the factors currently affecting adherence. Since 2010, there have been two SLRs (1, 8) reviewing non-adherence, the most recent of which was published in 2018. We initiated this SLR to capture the most recent data on adherence in the literature. The studies that met the inclusion criteria for this SLR included those with small (30

TABLE 2 | Recommendations for improving adherence.

Interaction with the healthcare team
Age-appropriate education and awareness of treatment objectives and the importance of adherence, particularly when administration shifts from caregiver to child (16, 29, 31)
Increased patient engagement, such as in selecting the device (16, 30) and in shared decision-making (11)
Ongoing feedback of treatment efficacy, to encourage compliance (16)
Adherence reminder tools
Electronic injection reminders, mobile phone reminders, and applications (13)
Gamified interventions that include goal setting, incentive-based engagement, and education (13)
Real-time monitoring of adherence using internet-connected devices (13)
Use of an electronic monitoring device to track adherence (12, 13, 23)
Device
Improvements in device design that reduce pain (29)/needle anxiety (32) and increase convenience (16)
Product
A product that does not require cold storage (i.e., storage-flexible rhGH) (21)
Reduction in the number of injections from daily to weekly (14)

rhGH, recombinant human growth hormone.

patients) and large (up to >13,000 patients) sample sizes, and the mean age of patients in the studies ranged from 6 years to 12.3 years. Although studies in other chronic conditions such as MS, JIA, or IBD were eligible for inclusion in this review, of the studies that met the inclusion criteria, only one study was in an indication (MS) outside of GHD. While this SLR is focused primarily on GHD, it is possible that the motivation for taking an injectable therapy may differ between patients with GHD and those with a chronic inflammatory condition. A key strength of this SLR was the fact that the included studies spanned a large number of different countries in several geographic regions, including the Americas, Europe, Asia, and the Middle East. This SLR is also one of the first to evaluate adherence in patients receiving the next generation of long-acting GH treatments. Based on the included studies, the overall trend observed was that pediatric patients had a high adherence to rhGH therapy. However, this observation should be interpreted in light of the limitations of this SLR.

One of the main limitations of this SLR was the difficulty in comparing studies, due to the different methods used for measuring and defining adherence. Although most studies reported adherence over 12 months, a few studies reported adherence over a shorter time period or failed to report the observation period, further limiting comparability among studies. More than half of the studies did not report mean or median adherence, and for the studies with categorical measures, the category definitions and cut-offs varied based on the study. Another potential limitation is the possible influence of the Hawthorne effect on the study findings; that is, patients knowing they were in a study may have been more likely to engage in desired behavior (adherence) than if they were not monitored. The fact that a risk-of-bias assessment was not performed on the single Chinese publication identified in the targeted search was also a study limitation.

From the 11 studies reporting 12-month mean adherence, adherence ranged from 73.3% (29) to 95.3% (24); the population-weighted mean adherence across the 11 studies was 79.3%. As stated above, of these 11 studies, the 2019 study by Farfel et al. was different from the others because adherence was based on the number of months with a pharmacy fill, rather than days' supply (29). This method of measurement may have underestimated patients' access to rhGH in the event that they received doses from sources other than through their pharmacy benefit (e.g., from a physician). This method may have also over-reported adherence if there was an overlap whereby the months' supply of rhGH exceeded the prescribed amount. Further, although patients may have picked up or received their treatment, it cannot be assumed that all doses were injected.

A total of six rhGH studies reported the percentage of patients who missed <1 dose per week (>85% of prescribed doses injected or in possession); adherence according to this definition ranged from 51% (based on pharmacy fills) (31) to 100% (self-reported) among patients able to select their choice of injection device. The high adherence rate in the two clinical trials, which used diaries to self-report adherence, could have been influenced by a potential Hawthorne effect and may not be reflective of real-world adherence to rhGH. The high adherence associated with the use of injector devices may have also been influenced by patients' awareness that utilization data were being sent to their health care provider or to researchers. This suggests that the use of injector devices with data reviewed regularly by a health care provider may be an effective method to increase/improve adherence to treatment. Additional studies of adherence among patients using other device types are needed to clarify the influence of the Hawthorne effect and the ramifications for routine clinical practice.

Of interest is the study by Mohseni et al. in 2018, which compared different methods of reporting adherence (15). Based on the self-reported, eight-item Morisky Medication Adherence Scale, 56.7% of children (aged 2–12 years) and 57.9% of adolescents (13–19 years) had moderate-to-high adherence to rhGH (15). Using the "auto-compliance method," based on self-reported number of injections divided by the number of injections prescribed (with ≥80% of prescribed injections reported as received being considered "adherent"), adherence rates were 95.2% among children and 95.5% among adolescents (15). The large difference in adherence seen in this study illustrates how the choice of methods used to measure and define adherence can affect reported adherence. Similarly, in the two published SLRs on adherence to rhGH, adherence rates also varied widely depending on the measures and definitions used, from 18% to 95% in the 2013 review by Fisher and Acerini (1), and from 29% to 93% in Graham et al. (8).

The barriers to adherence (**Figure 5**) identified in this review were similar to those reported by Fisher et al. in 2013 (1). The previous SLR identified injection-related factors associated with non-adherence as being perceived difficulty of injections, lack of choice of injection device, short duration of prescriptions, and discomfort (1). Of the 20 modifiable factors identified in Graham et al. (8), those related to poor adherence to rhGH included injection-related pain and discomfort, poor administration technique, forgetting injections, disruption in supply of injections due to short duration of prescriptions, and being away from home (8). Based on our extensive experience in clinical practice, we suggest that dissatisfaction with treatment outcome, a lack of understanding of the consequences of missed doses, and poor knowledge and understanding of the disease condition also constitute substantial barriers to adherence. Non-modifiable risk factors to adherence such as gender, age, race, severity, and duration of disease may also affect the impact of modifiable risk factors and should be carefully considered in studies evaluating adherence.

The studies included in this review recommended multiple methods of improving adherence, focused in particular on device choice, patient and caregiver education and engagement, reminders, and utilization monitoring. The one study that compared adherence to daily *vs.* weekly rhGH also suggested that weekly dosing could improve adherence compared with daily dosing. The increasing emergence of long-acting rhGH treatments has the potential to improve adherence and treatment outcomes. One potential consideration for long-acting rhGH products is that the consequences of missing an injection are

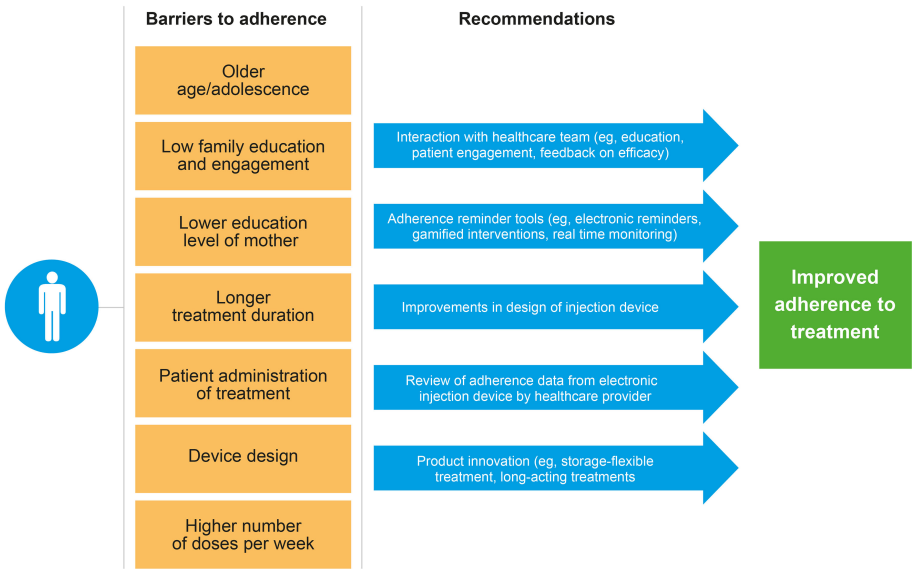


FIGURE 5 | Barriers to adherence and recommendations for improving adherence (identified in the included studies).

more severe, given that missing one injection is equivalent to missing seven daily rhGH injections. A number of study recommendations focused on technological innovations for the injection device, indicating great interest in the potential of technology to improve adherence. In addition to those already mentioned, features such as automatic recording of missed doses and automatic revision of subsequent doses (to compensate for a missed dose) may also help patients to remain adherent. The utilization of growth-prediction models (34) as part of patient consultation may also encourage adherence. Patients who fail to observe an adequate response to treatment may be less likely to adhere to treatment; optimizing treatment response using growth-prediction models may potentially help empower patients and reduce patient dissatisfaction with treatment, thereby encouraging patients to remain adherent. However, the use of prediction models to improve adherence requires further study. Clinicians should consider reassessing a patient's diagnosis if poor response is observed, particularly if treatment adherence has been high. There are several other interventions that focus more on behavior modification such as adherence therapy, cognitive behavioral therapy, cognitive adaptation training, family interventions, and psychoeducation/monetary-based interventions that have not been explored to date in the field of rhGH therapy. Furthermore, given that the previous studies have already identified the risk factors that predispose patients to impaired adherence, future studies should explore a more individualized approach where interventions could be targeted at those who are at a high risk of poor adherence.

Study Recommendations Based on SLR Findings

One of the main findings from this review was the substantial heterogeneity in the way adherence was defined, measured, and reported in recent publications on rhGH. This heterogeneity makes it difficult to compare studies and to uncover trends and patterns across the literature. Therefore, we propose the following recommendations to enable more effective characterization/comparison of adherence data across future studies and publications. First, we propose standardizing the way adherence is defined, measured, and reported. In addition, adoption of standardized study designs, including adherence measures, definitions of "adherent" *vs.* "non-adherent," and outcomes, is also recommended. Quantitative data describing the impact of missed doses on growth would also be valuable for determining the threshold of adherence to be reported. Furthermore, at a minimum, studies should report the presence or absence of any association between adherence and study duration, patient age, and rhGH indication. Lastly, in studies where the primary purpose is to study adherence, adherence should be measured using more than one method — agreement between different methods in the same study should be assessed. We believe that these measures should help address the substantial heterogeneity among the studies identified in the published literature.

CONCLUSIONS

Reported adherence was >80% in many studies, particularly in those that used an injector device or diaries for self-reporting; adherence among patients who are not being actively monitored or who are not participants in a study may be lower, due to the Hawthorne effect. Among the 22 rhGH studies included in this review of the published literature, there was substantial heterogeneity in how adherence to rhGH was defined, measured, and reported. Standardization of how adherence is

defined and reported, as well as in study design and outcomes measured, would enable more extensive comparisons to be made among different pediatric populations. Factors that could improve adherence include patient and caregiver education and active involvement in the treatment plan, including choice of device. Once available, long-acting rhGH formulations, which would allow weekly instead of daily dosing, may also result in improved adherence to treatment. It will be important to use proper comparisons of adherence between daily rhGH and long-acting rhGH preparations to demonstrate this relationship.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

RG, SFA, MM, DL, TT, and BSM contributed to the design, planning, and conception of the study, as well as data interpretation and manuscript development. DL (fluent in Chinese) conducted the literature search in the Wan Fang database. All authors reviewed manuscript drafts and have reviewed and approved the final version for submission.

FUNDING

This study was funded by Pfizer.

ACKNOWLEDGMENTS

Karen Smoyer (KS), PhD, and Catherine Rolland (CR), PhD, of Curo, conducted the Embase® and MEDLINE® database searches, screening, and data extraction, which was funded by Pfizer. Medical writing and editorial support were provided by Chu Kong Liew, PhD, of Engage Scientific Solutions and was funded by Pfizer.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.795224/full#supplementary-material

REFERENCES

1. Fisher BG, Acerini CL. Understanding the Growth Hormone Therapy Adherence Paradigm: A Systematic Review. *Horm Res Paediatr* (2013) 79:189–96. doi: 10.1159/000350251
2. Ahmed SF, Smith WA, Blamires C. Facilitating and Understanding the Family's Choice of Injection Device for Growth Hormone Therapy by Using Conjoint Analysis. *Arch Dis Child* (2008) 93:110–4. doi: 10.1136/adc.2006.105353
3. Cutfield WS, Derraik JG, Gunn AJ, Reid K, Delany T, Robinson E, et al. Non-Compliance With Growth Hormone Treatment in Children is Common and Impairs Linear Growth. *PloS One* (2011) 6:e16223. doi: 10.1371/journal.pone.0016223
4. De Pedro S, Murillo M, Salinas I, Granada ML, Martinez M, Puig-Domingo M, et al. Variability in Adherence to rhGH Treatment: Socioeconomic Causes and Effect on Children's Growth. *Growth Horm IGF Res* (2016) 26:32–5. doi: 10.1016/j.ghir.2015.12.002
5. Desrosiers P, O'Brien F, Blethen S. Patient Outcomes in the GHMonitor: The Effect of Delivery Device on Compliance and Growth. *Pediatr Endocrinol Rev* (2005) 2(Suppl 3):327–31.
6. Lustig RH. Optimizing Growth Hormone Efficacy: An Evidence-Based Analysis. *Horm Res* (2004) 62(Suppl 3):93–7. doi: 10.1159/000080506
7. Aydin BK, Aycan Z, Siklar Z, Berberoglu M, Ocal G, Cetinkaya S, et al. Adherence to Growth Hormone Therapy: Results of a Multicenter Study. *Endocr Pract* (2014) 20:46–51. doi: 10.4158/EP13194.OR
8. Graham S, Weinman J, Auyeung V. Identifying Potentially Modifiable Factors Associated With Treatment Non-Adherence in Paediatric Growth Hormone Deficiency: A Systematic Review. *Horm Res Paediatr* (2018) 90:221–7. doi: 10.1159/000493211
9. Garritty X, Gartlehner G, Kamel C, King VJ, Nussbaumer-Streit B, Stevens A, et al. *Cochrane Rapid Reviews. Interim Guidance From the Cochrane Rapid Reviews Methods Group* (2020). Available at: https://methods.cochrane.org/rapidreviews/sites/methods.cochrane.org.rapidreviews/files/public/uploads/cochrane_rr_-_guidance-23mar2020-final.pdf (Accessed 15 January, 2021).
10. Centre for Reviews and DIssemination. *Systematic Reviews: Crd's Guidance for Undertaking Reviews in Health Care* (2009). Available at: https://www.york.ac.uk/media/crd/Systematic_Reviews.pdf (Accessed 15 June, 2021).
11. Ghezzi A, Bianchi A, Baroncini D, Bertolotto A, Malucchi S, Bresciamorra V, et al. A Multicenter, Observational, Prospective Study of Self- and Parent-Reported Quality of Life in Adolescent Multiple Sclerosis Patients Self-Administering Interferon-Beta1a Using RebiSmart-The FUTURE Study. *Neurol Sci* (2017) 38:1999–2005. doi: 10.1007/s10072-017-3091-6
12. Arrabal Vela MA, Garcia Gijon CP, Pascual Martin M, Benet Gimenez I, Areas Del Aguila V, Munoz-Rodriguez JR, et al. Adherence to Somatotropin Treatment Administered With an Electronic Device. *Endocrinol Diabetes Nutr* (2018) 65:314–8. doi: 10.1016/j.endinu.2018.02.003
13. Koledova E, Tornincasa V, van Dommelen P. Analysis of Real-World Data on Growth Hormone Therapy Adherence Using a Connected Injection Device. *BMC Med Inform Decis Mak* (2020) 20:176. doi: 10.1186/s12911-020-01183-1
14. Sävendahl L, Battelino T, Brod M, Hojby Rasmussen M, Horikawa R, Juul RV, et al. Once-Weekly Somapacitan vs Daily GH in Children With GH Deficiency: Results From a Randomized Phase 2 Trial. *J Clin Endocrinol Metab* (2020) 105:e1847–61. doi: 10.1210/clinem/dgz310
15. Mohseni S, Heydari Z, Qorbani M, Radfar M. Adherence to Growth Hormone Therapy in Children and Its Potential Barriers. *J Pediatr Endocrinol Metab* (2018) 31:13–20. doi: 10.1515/jpem-2017-0157
16. Bagnasco F, Di Iorgi N, Roveda A, Gallizia A, Haupt R, Maghnie M, et al. Prevalence and Correlates of Adherence in Children and Adolescents Treated With Growth Hormone: A Multicenter Italian Study. *Endocr Pract* (2017) 23:929–41. doi: 10.4158/EP171786.OR
17. Cardinale GM, Pesce S, Ingletto D, Mariano M, Catucci A, Corciulo N, et al. Monitoring of Treatment Adherence With Easypod in Six Italian Centers: A Real-World Experience. *Minerva Endocrinol* (2019) 44:246–51. doi: 10.23736/S0391-1977.18.02843-2
18. Centonze C, Guzzetti C, Orlando G, Loche S, Italian EI. Adherence to Growth Hormone (GH) Therapy in Naive to Treatment GH-Deficient Children: Data of the Italian Cohort From the Easypod Connect Observational Study (ECOS). *J Endocrinol Invest* (2019) 42:1241–4. doi: 10.1007/s40618-019-01046-1
19. Loche S, Salerno M, Garofalo P, Cardinale GM, Licenziati MR, Citro G, et al. Adherence in Children With Growth Hormone Deficiency Treated With r-hGH and the Easypod Device. *J Endocrinol Invest* (2016) 39:1419–24. doi: 10.1007/s40618-016-0510-0

20. Maggio MC, Vergara B, Porcelli P, Corsello G. Improvement of Treatment Adherence With Growth Hormone by Easypod Device: Experience of an Italian Centre. *Ital J Pediatr* (2018) 44:113. doi: 10.1186/s13052-018-0548-z
21. Kappelgaard AM, Metzinger CP, Schnabel D. A Web-Based Survey Assessing the Impact of Storage Flexibility on the Daily Life of Patients and Caregivers Administering Growth Hormone. *Expert Rev Med Devices* (2015) 12:517–27. doi: 10.1586/17434440.2015.1069180
22. van Dommelen P, Koledova E, Wit JM. Effect of Adherence to Growth Hormone Treatment on 0-2 Year Catch-Up Growth in Children With Growth Hormone Deficiency. *PloS One* (2018) 13:e0206009. doi: 10.1371/journal.pone.0206009
23. Koledova E, Stoyanov G, Ovbude L, Davies PSW. Adherence and Long-Term Growth Outcomes: Results From the Easypod() Connect Observational Study (ECOS) in Paediatric Patients With Growth Disorders. *Endocr Connect* (2018) 7:914–23. doi: 10.1530/EC-18-0172
24. Rodriguez Arnao MD, Rodriguez Sanchez A, Diez Lopez I, Ramirez Fernandez J, Bermudez de la Vega JA, Yeste Fernandez D, et al. Adherence and Long-Term Outcomes of Growth Hormone Therapy With Easypod in Pediatric Subjects: Spanish ECOS Study. *Endocr Connect* (2019) 8:1240–9. doi: 10.1530/EC-19-0325
25. Blanco-Lopez A, Antillon-Ferreira C, Saavedra-Castillo E, Barrientos-Perez M, Rivero-Escalante H, Flores-Caloca O, et al. Adherence to Treatment in Children With Growth Hormone Deficiency, Small for Gestational Age and Turner Syndrome in Mexico: Results of the Easypod Connect Observational Study (ECOS). *J Endocrinol Invest* (2020) 43:1447–52. doi: 10.1007/s40618-020-01218-4
26. Charmandari E, Vlachopapadopoulou E, Kyritsi EM, Sakellariou D, Koledova E, Nespithal K, et al. Adherence and Long-Term Outcomes of Therapy in Paediatric Patients in Greece Using the Easypod Electromechanical Device for Growth Hormone Treatment: The Phase IV Multicentre Easypod Connect Observational Study (ECOS). *Growth Horm IGF Res* (2020) 53-54:101336. doi: 10.1016/j.ghir.2020.101336
27. Chung WY, Yoo HW, Hwang JS, Ko CW, Kim HS, Jin DK, et al. Effect of Growth Hormone Therapy on Height Velocity in Korean Children With Idiopathic Short Stature: A Phase III Randomised Controlled Trial. *Horm Res Paediatr* (2018) 90:44–53. doi: 10.1159/000491016
28. Dumitrescu CP, Procopiuc C, Dumitriu N, Micle I, Anton M, Moisuc A. COMPLIA: A 12-Month Prospective, Multicentre, Non-Interventional Study to Evaluate Treatment Adherence and Treatment Satisfaction in a Growth Hormone Deficient Paediatric Population Treated With Nutropinaq® a Somatropin Analogue. *Acta Endocrinol (Buchar)* (2020) 16:192–8. doi: 10.4183/aeb.2020.192
29. Farfel A, Shalitin S, Morag N, Meyerovitch J. Long-Term Adherence to Growth Hormone Therapy in a Large Health Maintenance Organization Cohort. *Growth Horm IGF Res* (2019) 44:1–5. doi: 10.1016/j.ghir.2018.10.004
30. Gau M, Takasawa K. Initial Patient Choice of a Growth Hormone Device Improves Child and Adolescent Adherence to and Therapeutic Effects of Growth Hormone Replacement Therapy. *J Pediatr Endocrinol Metab* (2017) 30:989–93. doi: 10.1515/jpem-2017-0146
31. Lass N, Reinehr T. Low Treatment Adherence in Pubertal Children Treated With Thyroxin or Growth Hormone. *Horm Res Paediatr* (2015) 84:240–7. doi: 10.1159/000437305
32. Michaelidou M, Whitten S, Bajaj P, Knight A, Spoudeas HA. Improved Adherence and Growth Outcomes With Jet-Delivered Growth Hormone. *J Pediatr Endocrinol Metab* (2019) 32:207–13. doi: 10.1515/jpem-2018-0067
33. Li J, Liu D. Compliance Analysis of Children's GHD Treated With PEG rhGH. *World Latest Med Inf* (2020) 20:18–20.
34. Loftus J, Lindberg A, Aydin F, Gomez R, Maghnie M, Rooman R, et al. Individualised Growth Response Optimisation (iGRO) Tool: An Accessible and Easy-to-Use Growth Prediction System to Enable Treatment Optimisation for Children Treated With Growth Hormone. *J Pediatr Endocrinol Metab* (2017) 30:1019–26. doi: 10.1515/jpem-2017-0120

Conflict of Interest: RG: employee of and owns shares/options in Pfizer. MM: research support from Pfizer and Merck Serono and consultant for Pfizer, Novo Nordisk, Merck Serono, Ferring, Biomarin, and Ascendis. SFA: unrestricted research and education support from Diurnal, Neurocrine Biosciences, Novo Nordisk; chief investigator for Acerus; and consultant for Sanofi. TT: consultant for JCR pharmaceuticals. BSM: consultant for AbbVie, Ascendis Pharma, BioMarin, Merck Serono, Novo Nordisk, Orchard Therapeutics, Pfizer, Sandoz, Tolmar and Vertice Pharma and research support from Alexion, Abbvie, Amgen, Lumos Pharma, Novo Nordisk, OPKO, and Pfizer.

The authors declare that this study received funding from Pfizer. The funder had the following involvement in the study: input into study design, interpretation of data, and preparation of the manuscript. The authors had final authority on all aspects of the manuscript content and development, including on the choice of journal.

The reviewer CG declared a past co-authorship with several of the authors (MM and SFA) to the handling editor.

frontiers | Frontiers in Pediatrics

ORIGINAL RESEARCH
published: 08 April 2022
doi: 10.3389/fped.2022.829372

A Study to Evaluate Accuracy and Validity of the EFAI Computer-Aided Bone Age Diagnosis System Compared With Qualified Physicians

*Chi-Fung Cheng[1], Ken Ying-Kai Liao[2], Kuan-Jung Lee[2] and Fuu-Jen Tsai[3]**

[1] Big Data Center, China Medical University Hospital, Taichung City, Taiwan, [2] Ever Fortune.AI Co., Ltd., Taichung City, Taiwan, [3] Department of Medical Genetics, China Medical University Hospital, Taichung City, Taiwan

OPEN ACCESS

Edited by:
M. Savage,
Queen Mary University of London,
United Kingdom

Reviewed by:
Giorgio Radetti,
Ospedale di Bolzano, Italy
Fergus Cameron,
Royal Children's Hospital, Australia

****Correspondence:***
Fuu-Jen Tsai
d0704@mail.cmuh.org.tw

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: *05 December 2021*
Accepted: *25 February 2022*
Published: *08 April 2022*

Citation:
Cheng C-F, Liao KY-K, Lee K-J and Tsai F-J (2022) A Study to Evaluate Accuracy and Validity of the EFAI Computer-Aided Bone Age Diagnosis System Compared With Qualified Physicians. Front. Pediatr. 10:829372. doi: 10.3389/fped.2022.829372

Study Objectives: In previous research, we built a deep neural network model based on Inception-Resnet-v2 to predict bone age (EFAI-BAA). The primary objective of the study was to determine if the EFAI-BAA was substantially concordant with the qualified physicians in assessing bone ages. The secondary objective of the study was to determine if the EFAI-BAA was no different in the clinical rating (advanced, normal, or delayed) with the qualified physicians.

Method: This was a retrospective study. The left-hand X-ray images of male subjects aged 3–16 years old and female subjects aged 2–15 years old were collected from China Medical University Hospital (CMUH) and Asia University Hospital (AUH) retrospectively since the trial began until the included image amount reached 368. This was a blinded study. The qualified physicians who ran, read, and interpreted the tests were blinded to the values assessed by the other qualified physicians and the EFAI-BAA.

Results: The concordance correlation coefficient (CCC) between the EFAI-BAA (EFAI-BAA), the evaluation of bone age by physician in Kaohsiung Veterans General Hospital (KVGH), Taichung Veterans General Hospital (TVGH2), and in Taipei Tzu Chi Hospital (TZUCHI-TP) was 0.9828 (95% CI: 0.9790–0.9859, p-value = 0.6782), 0.9739 (95% CI: 0.9681–0.9786, p-value = 0.0202), and 0.9592 (95% CI: 0.9501–0.9666, p-value = 0.4855), respectively.

Conclusion: There was a consistency of bone age assessment between the EFAI-BAA and each one of the three qualified physicians (CCC = 0.9). As the significant difference in the clinical rating was only found between the EFAI-BAA and the qualified physician in TVGH2, the performance of the EFAI-BAA was considered similar to the qualified physicians.

Keywords: bone age assessment, artificial intelligence, deep learning, concordance correlation coefficient (CCC), clinical practice

BACKGROUND

In pediatrics, the interpretation of bone age can accurately assess the maturity of an individual, and can also be used as a reference for the diagnosis of endocrine disorders in children (1). The well-known manual methods for bone age assessment are Greulich and Pyle (GP method) (2) and Tanner-Whitehouse (TW method) (3). The assessments are based on visual inspection or scoring and are characterized by intra- or extra-observer variability (4, 5). External variability is the difference in judgment standards or differences in the level of interpretation experience among physicians; internal variability is the possible difference in interpretation of the same image by the same physician at different times (6). In addition, the average interpretation time of the GP method in the past study was 1.4 min and TW method was 7.9 min. Both of these methods invisibly increase the time cost of physician visits (7).

In view of the rapid development of artificial intelligence in recent years, image recognition systems developed based on deep learning technology are becoming more and more mature in clinical applications. In the previous research, we introduced the Inception-Resnet-v2 neural network that was pre-trained on ImageNet database, from which to extract features as the basic model (8). At each bone age assessment, the radiologist compares the client's X-ray image to the GP reference image to assess their bone age and uses this as the ground truth for the model. Using training data from children and adolescents aged 2–18 in Taiwan, the network can predict well when given only the left hand bone X-ray and gender information. The purpose of this AI model is to reduce interpretation errors and actually reduce the complexity, time and cost of the bone age assessment process. The purpose of this research is to use the previously established deep learning model to examine the consistency and effectiveness of this model when it is actually put into clinical application scenarios.

MATERIALS AND METHODS

This was a blinded retrospective study. Since all recognizable information had been removed before data collection, no informed consent was required for this study. The qualified physicians who ran, read, and interpreted the tests were blinded to the values assessed by the other qualified physicians and the EFAI-BAA. This study was designed to evaluate the concordance of the EFAI-BAA in assessing bone ages, in comparison to each one of the three qualified physicians.

After the whole included images had been determined, the physicians received the data disk with all included images in and the guidance on how to use the electronic data capture (EDC) system. A physician had to fill in the bone age he/she assessed on the EDC after receiving the data disk. After the bone age corresponding to an image was filled in on the EDC, it might be changed with a rational explanation, and the process was recorded in the EDC. Only after all the physicians finished assessing all the allotted images, can the X-ray images be imported to the EFAI-BAA to get the bone ages inferred by the EFAI-BAA.

Study Design and Participants

The study subjects were selected from China Medical University Hospital (CMUH) and Asia University Hospital (AUH). Subjects were enrolled by using the following criteria. Inclusion criteria: (1) Male subjects aged 3 to 16 years old and female subjects aged 2–15 years old at the time of left-hand X-ray PA view image taking. (2) The image quality should be good enough for the physicians to evaluate the bone age. Exclusion criteria: (1) Subjects with skeletal dysplasia. (2) Subjects with congenital anomaly over the hand and wrist. (3) Any severe fracture over the hand and wrist that hindered the determination of the age. (4) Subjects with known malignancy of the left hand. The left-hand X-ray PA view images of male subjects aged 3–16 years old and female subjects aged 2–15 years old at the time when X-ray was taken were retrospectively provided by Medical record department. A total of 368 left-hand X-ray PA view images that met the inclusion/exclusion criteria from these studies were sequentially selected for the proposed study. The flowchart of the subject-selection process is presented in **Figure 1**.

Three independent certified qualified (with physician license) physicians from three centers in Taiwan, who were not part of the EFAI-BAA development, validation, or clinical study read the left-hand X-ray PA view images. Each of the three qualified physicians was provided with the same set of anonymized left-hand PA X-ray images. They assessed these left-hand X-ray PA view images manually and provide the bone age assessments in the EDC. The same set of left-hand X-ray PA view images were imported to the EFAI-BAA by an independent trained technician for bone age assessment. After the assessments were complete, the results were exported for the statistical analyses.

Imaging Filtering

In this study, the images were collected retrospectively from CMUH and AUH. A total of 368 DICOM files of left-hand PA view X-ray radiographs were collected (the number of images from either site should not be less than 30%). The information of the subject, which included gender, birth date, and examination date was acquired. At the time when the left-hand X-ray images were taken, the male subjects should be aged 3–16 years old and the female subjects should be aged 2–15 years old.

The X-ray images from Sep 1st, 2017 to Aug 31st, 2020 from CMUH and AUH were queried. The researcher should be used to conduct simple random sampling and provide the order of these random numbers using R (version 3.6.2). The researcher checked the basic information of the subjects including chronological age and gender based on the order and should assign the data to the corresponding age groups.

The expected number in each age group was shown in **Table 1**.

Screening

All the included images were burned into a data disk by the research assistant and provided to physicians, to examine the quality of every image. The criteria were (1) Complete left hand and wrist (the distal end of radius and ulna included). (2) The X-ray image of the left-hand PA view. (3) No shadow on the image (such as wearing a ring or a holding fist). (4) The edge of

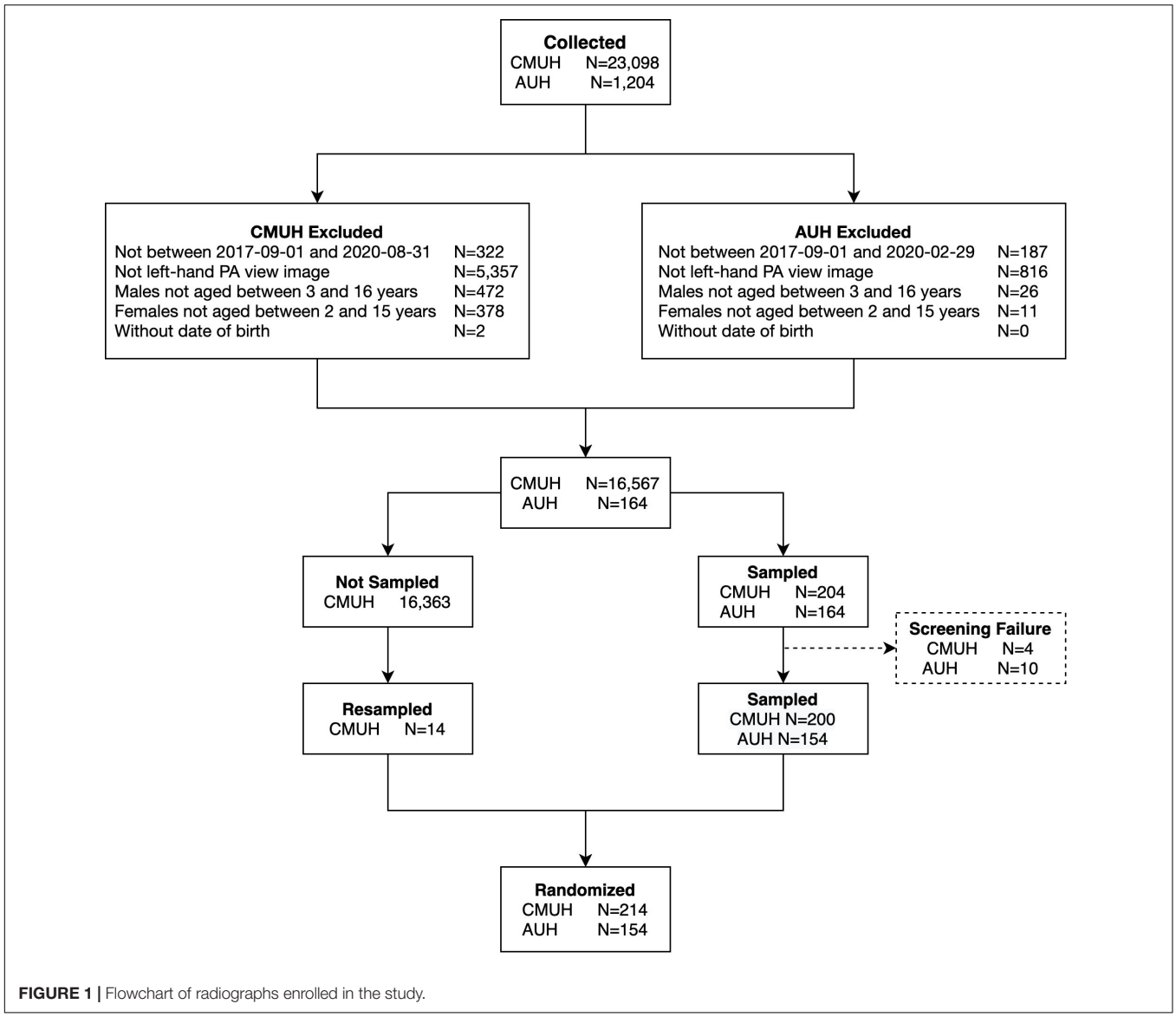


FIGURE 1 | Flowchart of radiographs enrolled in the study.

each bone including carpals and metacarpals should be seen and the size of the epiphyseal plate and the degree the epiphyseal plate merged with the bone should be distinguishable.

After the image quality was confirmed, subjects were eligible for enrollment in the study only if they met all the inclusion/exclusion criteria. Subsequently, the research assistant should log in to the EDC system with his/her account and should establish the eCRF for each subject being included after the filtering process. The following information should be entered into the corresponding column: gender, birth date, and the date X-ray taken.

Re-screening

After the screening process described above, the data amount might be insufficient since the disqualification was sifted. On that occasion, the process was repeated from checking each set of data in the order decided through simple random sampling, assigning the data to the age groups, to the image quality and data qualification screening. The process was repeated until the included amount reached the expected amount.

Bone Age Assessment

On each included X-ray image, a verification code (ckCode) was marked. Subsequently, the X-ray images along with gender were burned into the data disk, followed by providing two duplicate disks to physicians who participated in this trial. The physicians evaluated the bone age of each image according to the GP method. The physicians logged in to the EDC system with their accounts and passwords. The physicians keyed in the ckCode and corresponding bone age of the image on the eCRF. Only after confirming all the participating physicians had finished evaluating, the included images were imported into the EFAI-BAA by the research assistant to get the bone ages inferred by the medical device for the test.

Statistical Analysis

The agreement between the EFAI-BAA and each one of the three qualified physicians was assessed using the concordance correlation coefficient (CCC) statistical analysis method (9). The performance of the EFAI-BAA was validated when the concordance criterion between the EFAI-BAA and each one of the three qualified physicians was met. The clinical rating assessed by the EFAI-BAA and the qualified physicians was considered, and the Chi-square test was used to determine the difference in the clinical rating between the EFAI-BAA and each one of the three qualified physicians. The accuracy of the EFAI-BAA compared to each one of the three qualified physicians was calculated as well. The performance of the EFAI-BAA was evaluated by the Root Mean Square (RMS) and Mean Absolute Deviation (MAD) of bone age assessment between the EFAI-BAA and each one of the three qualified physicians. The paired *t*-test was used to compare the mean difference in bone age assessment between the EFAI-BAA and each one of the three qualified physicians. The Bland-Altman plot was created for displaying the difference in bone age assessment between the EFAI-BAA and each one of the three qualified physicians (**Supplementary Figures 1–3**). For general consideration, descriptive statistics for categorical variables included the number of subjects and percentage; descriptive statistics for continuous variables included the number of observations, mean, SD, median, minimum, and maximum values.

TABLE 1 | Summary of baseline characteristics.

Gender	Statistics	Pre-puberty[a]	Early and mid-puberty[b]	Late puberty[c]	Overall
Male	N (%)	94 (25.54%)	66 (17.93%)	30 (8.15%)	190 (51.63%)
	Mean	6.23	11.48	15.12	9.46
	Median	6.34	11.46	15.08	9.27
	SD	1.76	1.19	0.50	3.71
	Min	3.02	9.08	14.12	3.02
	Max	8.93	13.84	15.99	15.99
Female	N (%)	77 (20.92%)	71 (19.29%)	30 (8.15%)	178 (48.37%)
	Mean	4.86	10.05	13.85	8.44
	Median	5.11	10.31	13.73	8.43
	SD	1.56	1.49	0.61	3.68
	Min	2.06	7.04	13.01	2.07
	Max	6.99	12.91	14.92	14.92
Total	N (%)	171 (46.47%)	137 (37.23%)	60 (16.30%)	368 (100.00%)
	Mean	5.61	10.74	14.49	8.97
	Median	5.71	10.84	14.65	8.79
	SD	1.80	1.53	0.85	3.73
	Min	2.06	7.04	13.01	2.06
	Max	8.93	13.84	15.99	15.99

[a] *Pre-puberty (Female: CA 2–7 years old; Male: CA 3–9 years old).*
[b] *Early and Mid-puberty (Female: CA 7–13 years old; Male: CA 9–14 years old).*
[c] *Late Puberty (Female: CA 13–15 years old; Male: CA 14–16 years old).*
Abbreviation: CA, Chronological Age.

RESULTS

In this study, the images were collected retrospectively from CMUH and AUH. A total of 368 DICOM files of left-hand PA view X-ray radiographs were collected (the number of images from either site should not be less than 30%). The information of the subject, which included gender, birth date, and date of examination, was acquired. The results of the physicians' assessments were compared against the bone age assessments by the EFAI-BAA.

The primary endpoint for the study was the bone ages assessed by the EFAI-BAA and the qualified physicians. The analysis result of the primary endpoint was presented in **Table 2**. The CCC between EFAI-BAA and KVGH (#1) was 0.98 (0.98, 0.99); the CCC between EFAI-BAA and TVGH2 (#2) was 0.97 (0.97, 0.98); the CCC between EFAI-BAA and TZUCHI-TP (#3) was 0.96 (0.95, 0.97).

The secondary endpoint was the clinical rating assessed by the EFAI-BAA and the qualified physicians. By calculating the 95% interval of the normal bone age distribution by the mean bone age ± 2SD, the bone age assessed would fall within the normal range (normal), out of the upper side of the normal range (advanced), or out of the lower side of the normal range (delayed). The analysis result of the secondary endpoint was presented in **Table 3**. The number and percentage of "Advanced," "Normal," and "Delayed" for EFAI-BAA was 38 (10.33%), 249 (67.66%), and 81 (22.01%), respectively ($p = 0.6782$); for KVGH (#1) was 35 (9.51%), 260 (70.65%), and 73 (19.84%), respectively; for TVGH2 (#2) was 49 (13.32%), 266 (72.28%), and 53 (14.40%), respectively ($p = 0.0202$); and, for TZUCHI-TP (#3) was 41 (11.14%), 259 (70.38%), and 68 (18.48%), respectively ($p = 0.4855$).

The accuracy of the EFAI-BAA was presented in **Table 4**. The accuracy of EFAI-BAA compared to KVGH (#1) in the pre-puberty, early and mid-puberty, and late puberty group, and the overall age groups was 76.02, 81.02, 93.33, and 80.71%, respectively; the accuracy of EFAI-BAA compared to TVGH2 (#2) in the pre-puberty, early and mid-puberty, and late puberty group, and the overall age groups was 70.76, 86.13, 95.00, and 80.43%, respectively; the accuracy of EFAI-BAA compared to TZUCHI-TP (#3) in the pre-puberty, early and mid-puberty, and late puberty group, and the overall age groups were 66.67, 77.37, 96.67, and 75.54%, respectively.

The RMS and MAD and paired *t*-test of bone age assessment in each age group were presented in **Table 5**. The RMS (MAD) between EFAI-BAA and KVGH (#1) in the pre-puberty, early and mid-puberty, and late puberty group, and the overall age

TABLE 2 | Differences in the CCC scores (primary endpoint) between three physicians and EFAI-BAA.

Reference	CCC* (95% CI)		
	KVGH (#1)	TVGH2 (#2)	TZUCHI-TP (#3)
EFAI-BAA	0.98 (0.98, 0.99)	0.97 (0.97, 0.98)	0.96 (0.95, 0.97)

**Concordance correlation coefficient (CCC).*

TABLE 3 | Differences in the clinical rating (secondary endpoint) between three physicians and EFAI-BAA.

Site	Clinical rating			Total	*P*-value[a]	*P*-value[b]
	Advanced	Normal	Delayed			
EFAI-BAA	38 (10.33%)	249 (67.66%)	81 (22.01%)	368 (100.00%)	0.157	ref.
KVGH (#1)	35 (09.51%)	260 (70.65%)	73 (19.84%)	368 (100.00%)		0.6782
TVGH2 (#2)	49 (13.32%)	266 (72.28%)	53 (14.40%)	368 (100.00%)		0.0202
TZUCHI-TP (#3)	41 (11.14%)	259 (70.38%)	68 (18.48%)	368 (100.00%)		0.4855

[a]*Chi-square test of the difference in the clinical rating among the EFAI-BAA and the three qualified physicians.*
[b]*Chi-square test of the difference in the clinical rating between the EFAI-BAA and each of the three qualified physicians.*

groups was 0.81 (0.62), 0.75 (0.60), 1.02 (0.92), and 0.82 (0.66), respectively ($p = 0.0889$); the RMS (MAD) between EFAI-BAA and TVGH2 (#2) in the pre-puberty, early and mid-puberty, and late puberty group, and the overall age groups was 1.22 (0.90), 0.73 (0.56), 0.89 (0.76), and 1.01 (0.75), respectively ($p < 0.0001$); the RMS (MAD) between EFAI-BAA and TZUCHI-TP (#3) in the pre-puberty, early and mid-puberty, and late puberty group, and the overall age groups was 1.19 (0.94), 1.46 (0.88), 0.87 (0.74), and 1.25 (0.89), respectively ($p = 0.2206$).

DISCUSSION

This retrospective study evaluated the accuracy and efficiency of AI system developed for automatic bone age assessment of children in Taiwan. The results show that compared with EFAI-BAA in manually assessed bone age based on the Greulich-Pyle method by three physicians from different hospitals, regardless of gender, this AI model can obtain a highly consistent and accurate bone age assessment by automatically analyzing X-rays of the left wrist.

The bone age assessment of KVGH (#1) was highly consistent with EFAI-BAA in the CCC and the distribution of clinical rating (**Tables 2**, **3**). The bone age assessment of TVGH2 (#2) was averagely higher than that of EFAI-BAA, thus the mean of bone age assessment of TVGH2 (#2) was significantly different from that of EFAI-BAA (**Table 5**), and the distribution of clinical rating of TVGH2 (#2) was slightly shifted to the grade of "Advanced" (**Table 3**). Although the divergence of bone age assessment of TZUCHI-TP (#3) was high, TZUCHI-TP (#3) was still similar to EFAI-BAA in the mean of bone age assessment and the distribution of clinical rating (**Tables 3**, **5**), respectively.

Because each lower bound of the two-sided 95% CI of the CCC between the EFAI-BAA and each one of the three qualified physicians was greater than 0.90, the three null hypotheses were all rejected, which meant there was a consistency of bone age assessment between the EFAI-BAA and each one of the three qualified physicians. As the significant difference in the clinical rating was only found between the EFAI-BAA and the qualified physician in TVGH2 (#2), the performance of the EFAI-BAA was considered similar to the qualified physicians.

In recent years, many studies have begun to try to use deep learning methods to assess bone age on left-hand x-ray images (10–16), and a well-trained AI bone age assessment system is as accurate as clinical experts. There was significant intra-individual variability of 0.94 vs. 0.74 years for the GP and TW methods, respectively (7). This variability can be reduced to 0.31 years through EFAI-BAA (8). Clinical diagnostic tools developed by deep learning models are often criticized because they cannot be explained intuitively (black box) (17–19). However, attribute to its excellent interpretation efficiency compared with traditional GP and TW methods, it has been proven to save more interpretation time for physicians (20).

The Greulich-Pyle method is used to assess the maturity of bone age and has been widely used. However, it should be noted that this method is established on Caucasian ethnicity and is highly dependent on the experience of radiologists. It's prone to cause bias when GP method was applied to different generations, races or specific age groups for bone age assessment (21–26). Similarly, due to this study was a retrospective design, all x-ray images were from the China Medical University Hospital and

TABLE 4 | Accuracy of the EFAI-BAA compared with different sites physicians.

Age group	Accuracy		
	EFAI-BAA vs. #1	EFAI-BAA vs. #2	EFAI-BAA vs. #3
Pre-puberty	76.02%	70.76%	66.67%
Early and mid-puberty	81.02%	86.13%	77.37%
Late puberty	93.33%	95.00%	96.67%
[-1.2pt] Overall	80.71%	80.43%	75.54%

#1, Kaohsiung Veterans General Hospital (KVGH);
#2, Taichung Veterans General Hospital (TVGH2);
#3, Taipei Tzu Chi Hospital (TZUCHI-TP).

TABLE 5 | Root mean square and mean absolute deviation of bone age assessment in each puberty group.

Site	Root mean square (mean absolute deviation)				*P*-value*
	Pre-puberty	Early and mid-puberty	Late puberty	Overall	
EFAI-BAA	ref.	ref.	ref.	ref.	ref.
KVGH (#1)	0.81 (0.62)	0.75 (0.60)	1.02 (0.92)	0.83 (0.66)	0.0889
TVGH2 (#2)	1.22 (0.90)	0.73 (0.56)	0.89 (0.76)	1.01 (0.75)	<0.0001
TZUCHI-TP (#3)	1.19 (0.94)	1.46 (0.88)	0.87 (0.74)	1.25 (0.89)	0.2206

**P-value: paired t-test of bone age assessment for the overall age groups between the EFAI-BAA and each one of the three qualified physicians.*

Asia University Hospital. Therefore, the accuracy of EFAI-BAA has yet to be evaluated in different races or children who were less than 2 years old or over 16 years old. Finally, although there is no statistically significant difference in the assessment between EFAI-BAA and the three clinicians, it does not substitute the doctor's clinical decision-making, and can only provide the doctor with clinical assistance. EFAI-BAA only predicts the bone age based on the information provided by the images and lacks other clinical information and other physiological factors of the patient.

CONCLUSION

In our study, it was shown that there was no statistically significant difference between bone age assessment of EFAI-BAA and three physicians from different sites in Taiwan. In addition, our results show that the AI-based bone age assessment system greatly reduces the time of interpreting bone age by physician compared with the Greulich-Pyle method. It can improve the efficiency of routine clinical examinations without affecting the accuracy of the assessment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the China Medical University Hospital Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

F-JT had the idea and designed the study and responsible for acquisition of data. C-FC, KY-KL, and K-JL analyzed and interpreted the data and provided administrative, technical, logistical or material support. C-FC drafted the article and submitted the manuscript for publication. F-JT and C-FC critically revised the manuscript for important intellectual contents. All authors had the final approval of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.829372/full#supplementary-material

Supplementary Figure 1 | The Bland-Altman plot for EFAI-BAA vs. KVGH (#1).

Supplementary Figure 2 | The Bland-Altman plot for EFAI-BAA vs. TVGH2 (#2).

Supplementary Figure 3 | The Bland-Altman plot for EFAI-BAA vs. TZUCHI-TP (#3).

REFERENCES

1. Gilsanz V, Ratib O. *Hand Bone Age: A Digital Atlas of Skeletal Maturity*. Heidelberg: Springer Science & Business Media (2005).
2. Greulich WW, Pyle SI. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*. Redwood City, CA: Stanford University Press (1959).
3. Tanner JM. *Assessment of Skeletal Maturity and Prediction of Adult Height: TW 2 Method*. San Diego, CA: Academic Press (1983).
4. Cox LA. Tanner-Whitehouse method of assessing skeletal maturity: problems and common errors. *Horm Res.* (1996) 45:53–5. doi: 10.1159/000184848
5. Bull R, Edwards P, Kemp P, Fry S, Hughes I. Bone age assessment: a large scale comparison of the Greulich and Pyle, and Tanner and Whitehouse (TW2) methods. *Arch Dis Childh.* (1999) 81:172–3. doi: 10.1136/adc.81.2.172
6. Roche A, Rohmann CG, French NY, Daìvila GH. Effect of training on replicability of assessments of skeletal maturity (Greulich-Pyle). *Am J Roentgenol.* (1970) 108:511–5. doi: 10.2214/ajr.108.3.511
7. King D, Steventon D, O'sullivan M, Cook A, Hornsby V, Jefferson I, et al. Reproducibility of bone ages when performed by radiology registrars: an audit of Tanner and Whitehouse II versus Greulich and Pyle methods. *Br J Radiol.* (1994) 67:848–51. doi: 10.1259/0007-1285-67-801-848
8. Cheng CF, Huang ET-C, Kuo J-T, Liao KY-K, Tsai FJ. Report of clinical bone age assessment using deep learning for an Asian population in Taiwan. *Biomedicine.* (2021) 11:50–8. doi: 10.37796/2211-8039.1256
9. Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics.* (1989) 45:255–68. doi: 10.2307/2532051
10. Lee K-C, Lee K-H, Kang CH, Ahn K-S, Chung LY, Lee J-J, et al. Clinical validation of a deep learning-based hybrid (Greulich-Pyle and Modified Tanner-Whitehouse) method for bone age assessment. *Korean J Radiol.* (2021) 22:2017–25. doi: 10.3348/kjr.2020.1468
11. Wang F, Gu X, Chen S, Liu Y, Shen Q, Pan H, et al. Artificial intelligence system can achieve comparable results to experts for bone age assessment of Chinese children with abnormal growth and development. *PeerJ.* (2020) 8:e8854. doi: 10.7717/peerj.8854
12. Booz C, Yel I, Wichmann JL, Boettger S, Al Kamali A, Albrecht MH, et al. Artificial intelligence in bone age assessment: accuracy and efficiency of a novel fully automated algorithm compared to the Greulich-Pyle method. *Eur Radiol Exp.* (2020) 4:6. doi: 10.1186/s41747-019-0139-9
13. Lee H, Tajmir S, Lee J, Zissen M, Yeshiwas BA, Alkasab TK, et al. Fully automated deep learning system for bone age assessment. *J Digit Imaging.* (2017) 30:427–41. doi: 10.1007/s10278-017-9955-8
14. Han Y, Wang G. Skeletal bone age prediction based on a deep residual network with spatial transformer. *Comput Methods Programs Biomed.* (2020) 197:105754. doi: 10.1016/j.cmpb.2020.105754
15. Tong C, Liang B, Li J, Zheng Z. A deep automated skeletal bone age assessment model with heterogeneous features learning. *J Med Syst.* (2018) 42:249. doi: 10.1007/s10916-018-1091-6
16. Gao Y, Zhu T, Xu X. Bone age assessment based on deep convolution neural network incorporated with segmentation. *Int J Comput Assist Radiol Surg.* (2020) 15:1951–62. doi: 10.1007/s11548-020-02266-0
17. Park SH. Artificial intelligence in medicine: beginner's guide. *J Korean Soc Radiol.* (2018) 78:301–8. doi: 10.3122/jabfm.2022.01.210226
18. Castelvecchi D. Can we open the black box of AI? *Nat News.* (2016) 538:20. doi: 10.1038/538020a
19. Poon AI, Sung JJ. Opening the black box of AI-Medicine. *J Gastroenterol Hepatol.* (2021) 36:581–4. doi: 10.1111/jgh.15384
20. Dallora AL, Anderberg P, Kvist O, Mendes E, Diaz Ruiz S, Sanmartin Berglund J. Bone age assessment with various machine learning techniques: a systematic literature review and meta-analysis. *PLoS One.* (2019) 14:e0220242. doi: 10.1371/journal.pone.0220242
21. Maggio A, Flavel A, Hart R, Franklin D. Assessment of the accuracy of the Greulich and Pyle hand-wrist atlas for age estimation in a contemporary

Australian population. *Aust J Forensic Sci.* (2018) 50:385–95. doi: 10.1080/00450618.2016.1251970
22. Moradi M, Sirous M, Morovatti P. The reliability of skeletal age determination in an Iranian sample using Greulich and Pyle method. *Forensic Sci Int.* (2012) 223:372.e1–4. doi: 10.1016/j.forsciint.2012.08.030
23. udia Santos C, Ferreira M, Alves FC, nia Cunha E. Comparative study of greulich and pyle atlas and maturos 4.0 program for age estimation in a Portuguese sample. *Forensic Sci Int.* (2011) 212:276.e1–7. doi: 10.1016/j.forsciint.2011.05.032
24. Patil ST, Parchand M, Meshram M, Kamdi N. Applicability of greulich and pyle skeletal age standards to Indian children. *Forensic Sci Int.* (2012) 216:200.e1–4. doi: 10.1016/j.forsciint.2011.09.022
25. Büken B, Şafak A, Yazici B, Büken E, Mayda AS. Is the assessment of bone age by the Greulich-Pyle method reliable at forensic age estimation for Turkish children. *Forensic Sci Int.* (2007) 173:146–53. doi: 10.1016/j.forsciint.2007.02.023
26. Cavallo F, Mohn A, Chiarelli F, Giannini C. Evaluation of bone age in children: a mini-review. *Front Pediatr.* (2021) 9:580314. doi: 10.3389/fped.2021.580314

Conflict of Interest: KY-KL and K-JL were employed by Ever Fortune.AI Co., Ltd., Taichung, Taiwan.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Pediatrics

ORIGINAL RESEARCH
published: 22 April 2022
doi: 10.3389/fped.2022.866295

Association Between Recombinant Growth Hormone Therapy and All-Cause Mortality and Cancer Risk in Childhood: Systematic Review and Meta-Analysis

Mengyang He[1†], *Xiangling Deng*[1†], *Xuan Wang*[2†], *Yuxiang Wan*[1], *Jinchang Huang*[2*], *Zhixin Zhang*[3*] and *Wenquan Niu*[4*]

[1] Graduate School, Beijing University of Chinese Medicine, Beijing, China, [2] Beijing University of Chinese Medicine Third Affiliated Hospital, Beijing, China, [3] Department of International Medical, China-Japan Friendship Hospital, Beijing, China, [4] Institute of Clinical Medical Sciences, China-Japan Friendship Hospital, Beijing, China

Objectives: The safety of recombinant human growth hormone (rhGH) treatment in childhood and the role of rhGH therapy in promoting tumorigenesis and progression have been the subject of debate for decades. We aimed to systematically assess the relationship between rhGH therapy in children and adolescents and clinical outcomes, including all-cause mortality, cancer mortality, cancer incidence, and risk of the second neoplasm.

Methods: Literature retrieval, study selection, and data extraction were completed independently and in duplicate. Effect-size estimates are expressed as standardized mortality ratios (SMRs), standardized incidence ratio (SIR), and relative risk (RR) with a 95% CI.

Results: Data from 24 articles, involving 254,776 persons, were meta-analyzed. Overall analyses revealed the association of rhGH therapy was not statistically significant with all-cause mortality (SMR = 1.28; 95% CI: 0.58–2.84; $P = 0.547$; $I^2 = 99.2\%$; $Tau^2 = 2.154$) and cancer mortality (SMR = 2.59; 95% CI: 0.55–12.09; $P = 0.228$; $I^2 = 96.7\%$; $Tau^2 = 2.361$) and also cancer incidence (SIR = 1.54; 95% CI: 0.68–3.47; $P = 0.229$; $I^2 = 97.5\%$; $Tau^2 = 2.287$), yet statistical significance was observed for second neoplasm (RR = 1.77; 95% CI: 1.33–2.35; $P = 0.001$; $I^2 = 26.7\%$; $Tau^2 = 0.055$). Differences in the geographic region, gender, treatment duration, mean rhGH dose, overall rhGH exposure dose, and initial disease accounted for heterogeneity in the subgroup analyses.

Conclusion: Our findings indicate that the rhGH therapy is not related to all-cause mortality and cancer mortality and cancer incidence, yet it seems to trigger a second tumor risk. Future prospective studies are needed to confirm our findings and answer the more challenging question regarding the optimal dose of rhGH therapy in children and adolescents.

Keywords: mortality, children, cancer, medication safety, rhGH

OPEN ACCESS

Edited by:
M. Savage,
Queen Mary University of London,
United Kingdom

Reviewed by:
Lorenzo Iughetti,
University of Modena and Reggio Emilia, Italy
Pasquale Dolce,
University of Naples Federico II, Italy

***Correspondence:**
Wenquan Niu
niuwenquan_shcn@163.com
Zhixin Zhang
zhangzhixin032@163.com
Jinchang Huang
zryhhuang@163.com

[†] *These authors share first authorship*

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: *31 January 2022*
Accepted: *07 March 2022*
Published: *22 April 2022*

Citation:
He M, Deng X, Wang X, Wan Y, Huang J, Zhang Z and Niu W (2022) Association Between Recombinant Growth Hormone Therapy and All-Cause Mortality and Cancer Risk in Childhood: Systematic Review and Meta-Analysis. Front. Pediatr. 10:866295. doi: 10.3389/fped.2022.866295

INTRODUCTION

Since 1957, human growth hormone has been used to treat growth hormone deficiency and short stature, and it was supplanted by recombinant human growth hormone (rhGH) in 1985 (1). Initially, growth hormone was prescribed to patients with a severe growth hormone deficiency and its application is currently extended to children with short stature that is not primarily caused by an endogenous growth hormone deficiency, as well as to other scenarios, such as small for gestational age without catch-up growth or idiopathic short stature, Turner syndrome, short stature homeobox gene deficiency, Noonan syndrome, Prader–Willi syndrome, and growth failure associated with chronic renal insufficiency (2, 3). Generally, growth hormone therapy is considered to be safe, and serious adverse reactions rarely occur (4–6). However, in recent decades, the potential link between growth hormone therapy and tumor development or recurrence has gained increasing attention in clinical practice (7–11). In 2014, Deodati et al. (12) have undertaken a meta-analysis and reported that patients treated with growth hormone during childhood and adolescence had a significantly increased risk of all-cause mortality, cancer incidence, and second neoplasm after primary cancer. Contrastingly, in the to-date largest long-term follow-up study by Sävendahl et al., rhGH therapy was not associated with all-cause mortality in patients with isolated growth hormone deficiency or idiopathic short stature (13), and another large cohort study by Child et al. (2) also reported no significant association. In this context, the association between growth hormone therapy and all-cause mortality is still subject to an ongoing debate. With the accumulating publications afterward, there is a need to reexamine this association in a more comprehensive manner.

In an attempt to address this need and derive more reliable estimates, we performed an updated meta-analysis by pooling the results of both the prospective and retrospective cohorts in the medical literature to examine the association of rhGH therapy in children and adolescents with multiple clinical outcomes, including all-cause mortality, cancer mortality, cancer incidence, and risk of the second neoplasm. Another attempt was to identify the reasons for previous inconsistent reports, in other words, between-study heterogeneity.

METHODS

The performance of the meta-analysis has adhered to the guidelines in the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement (14). The PRISMA checklist is given in **Supplementary Table 1**.

This study is a meta-analysis of published studies; hence, ethical approval and informed consent are not needed.

Search Strategy

A literature search was conducted by reviewing the PubMed, MEDLINE, EMBASE, and Web of Science databases as of 6 November 2021. The following medical topic terms were used: (growth hormone or human growth hormone or somatotropin or somatropin or somatotrophin or GH or hGH or rhGH or rhGH or GH deficiency or growth hormone replacement therapy or GH replacement therapy) [Title] and (mortality or death or fatal or fatality or cancer or cancers or neoplasia or neoplasias or neoplasm or tumors or tumor or malignancy or malignancies or malignant neoplasm or CVD or cardiovascular disease) [Title/Abstract]. The reference lists of major retrieved articles were also manually searched to avoid potential missing hits.

The search process was independently conducted by two investigators (MH and XD) using the same medical topic terms. All the references retrieved were combined and duplicates were removed.

Inclusion/Exclusion Criteria

Our analysis was restricted to articles that met the following criteria: (1) study participants: women with BC; (2) endpoints: standardized mortality ratios (SMRs) or standardized incidence ratio (SIR) or relative risk (RR) with 95% CI; (3) study type: retrospective or prospective cohorts; (4) baseline exposure: growth hormone therapy; (5) follow-up rate: at least 70%; and (6) follow-up duration: $\geq$1 year. Articles were excluded if the involved study participants were adults or if they are case reports or case series, editorials, and narrative reviews.

Data Extraction

Two investigators (MH and XD) independently extracted data from each qualified article, including the first author, year of publication, the country where the study was conducted, sample size, study design, age at start rhGH therapy, rhGH dose, treatment duration, initial diagnosis, treatment duration, mean rhGH dose, overall exposure, effect estimation, and other confounding risk factors, if available. The divergence was resolved through a joint reevaluation of original articles, and if necessary, by a third author (WN).

Statistical Analyses

Data management was handled using the STATA software version 14.1 for Windows (Stata Corporation, College Station, Texas, USA). Effect-size estimates are expressed as SMR, SIR, or RR with 95% CI, where appropriate, and they are derived under the Mantel–Haenszel model. The difference between the two estimates was tested by the Z-test, as proposed by Altman and Bland (15). Pooled effect-size estimates were derived under a random-effects model, irrespective of the magnitude of between-study heterogeneity.

The inconsistency index (I^2) statistic, which represents the percent of diversity that is due to heterogeneity rather than chance, is used to quantify the magnitude of heterogeneity that was derived from a random-effects Mantel–Haenszel model. The I^2 >50% indicates the presence of significant heterogeneity and a higher percent corresponds to a higher degree of heterogeneity. Besides I^2 statistic, another index, τ^2 (Tau^2), was used to explore the sensitivity of the results to different levels of between-study heterogeneity. To account for possible sources of between-study heterogeneity from clinical and methodological aspects, a panel of prespecified subgroup analyses were performed according to geographic region, published year, study design, age at start rhGH

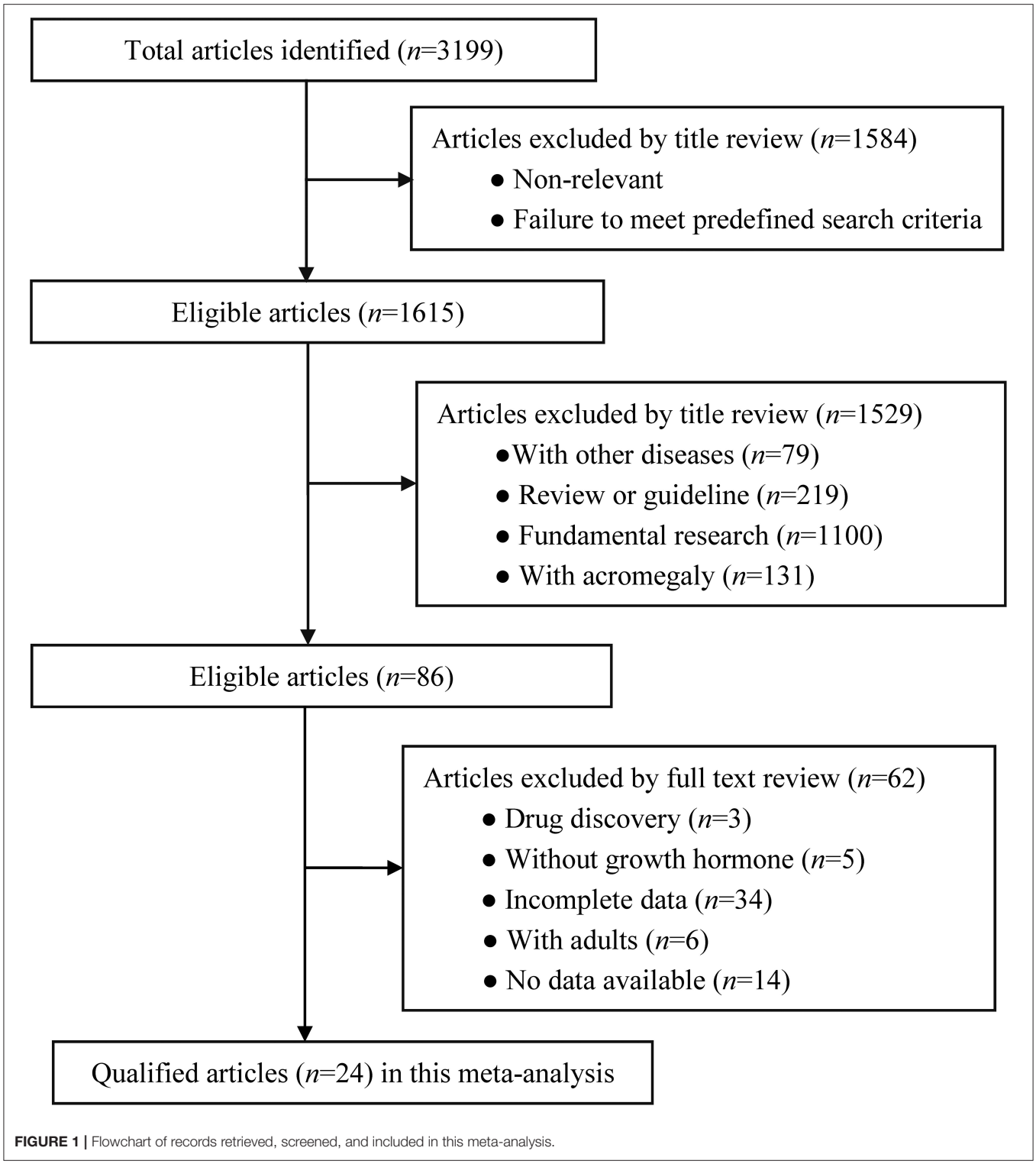


FIGURE 1 | Flowchart of records retrieved, screened, and included in this meta-analysis.

therapy, rhGH dose, treatment duration, initial diagnosis, mean rhGH dose, exposure, and follow-up interval, respectively.

The likelihood of publication bias was evaluated by both Begg's funnel plots and Egger's regression asymmetry tests at a significance level of 10%. The trim-and-fill method was also used to speculate the number of theoretically missing studies.

RESULTS

Eligible Studies

A total of 3,199 articles were initially identified after searching predefined public datasets according to subject terms, of which 24 met our eligibility criteria, including 2,54,776 children and

adolescents. The detailed selection process is shown in **Figure 1**. Among the eligible articles included, effect size estimates are expressed as SMR, SIR, and RR with 95% CIs.

Study Characteristics

Supplementary Table 2 shows the baseline characteristics of all the qualified articles in this meta-analysis. Of the 24 articles included, the outcome measure was all cause SMR in 7 articles (2, 13, 16–20), cancer SIR in 12 articles (1, 2, 5, 17, 19, 21–27), cancer SMR in 4 articles (16, 21, 25, 27), and second neoplasm in 8 articles (9, 10, 28–33). Only two articles (13, 16) presented data separately in boys and girls. Overall exposure of rhGH therapy was classified into four categories: <25, 25–50, 50–100, and ≥100 mg/kg.

Based on the previous medical history and physical health status, 3 articles (13, 16, 19) divided children into the low-risk, moderate-risk, and high-risk groups and 4 articles (5, 21, 22, 25) assorted children into the not-high-risk group.

Of the 24 qualified articles, two (13, 16) articles evaluated rhGH therapy duration <5 and ≥5 years. In total, eighteen articles were retrospective in design (5, 9, 10, 16, 18, 19, 21–24, 26–33) and 6 articles were prospective (1, 2, 13, 17, 20, 25). All the eligible articles were classified geographically into North America (5, 9, 10, 28), Asia (19), Europe (1, 13, 16–18, 21, 27, 29, 32, 33), and multinational (2, 20, 22–26, 30, 31).

Quality Assessment

Supplementary Table 3 shows the quality assessment of all the qualified articles by using the Newcastle–Ottawa Scale (NOS) tool for cohort studies. The average total score was 7.46 (range: 7–8), with an SD of 0.5.

Overall Analyses

After pooling the results of all the qualified articles, there was no statistical significance between rhGH therapy in childhood and all-cause mortality (SMR = 1.28; 95% CI: 0.58–2.84; $P = 0.547$; $I^2 = 99.2\%$; $Tau^2 = 2.154$), cancer mortality (SMR = 2.59; 95% CI: 0.55–12.09; $P = 0.228$; $I^2 = 96.7\%$; $Tau^2 = 2.361$), and standardized incidence ratio for cancer (SIR = 1.54; 95% CI: 0.68–3.47; $P = 0.229$; $I^2 = 97.5\%$; $Tau^2 = 2.287$). In contrast, there was a statistically significant association with the development of second neoplasm (RR = 1.77; 95% CI: 1.33–2.35; $P = 0.001$; $I^2 = 26.7\%$; $Tau^2 = 0.055$) (**Figure 2**).

Cumulative and Influential Analyses

In the cumulative analyses, included studies got completely similar conclusions consistently and trends tended to stabilize. The influential analyses revealed no significant impact of any single study on overall effect-size estimates.

Publication Bias

Figure 3 shows Begg's funnel plot and Egger's test for assessing publication bias of rhGH therapy with all-cause mortality, cancer mortality, the standardized incidence of cancer, and the occurrence of the second neoplasm.

Begg's funnel plots seemed symmetrical. As reflected by Egger's test, there was a low likelihood of publication bias for standardized incidence of cancer ($P = 0.525$) and occurrence

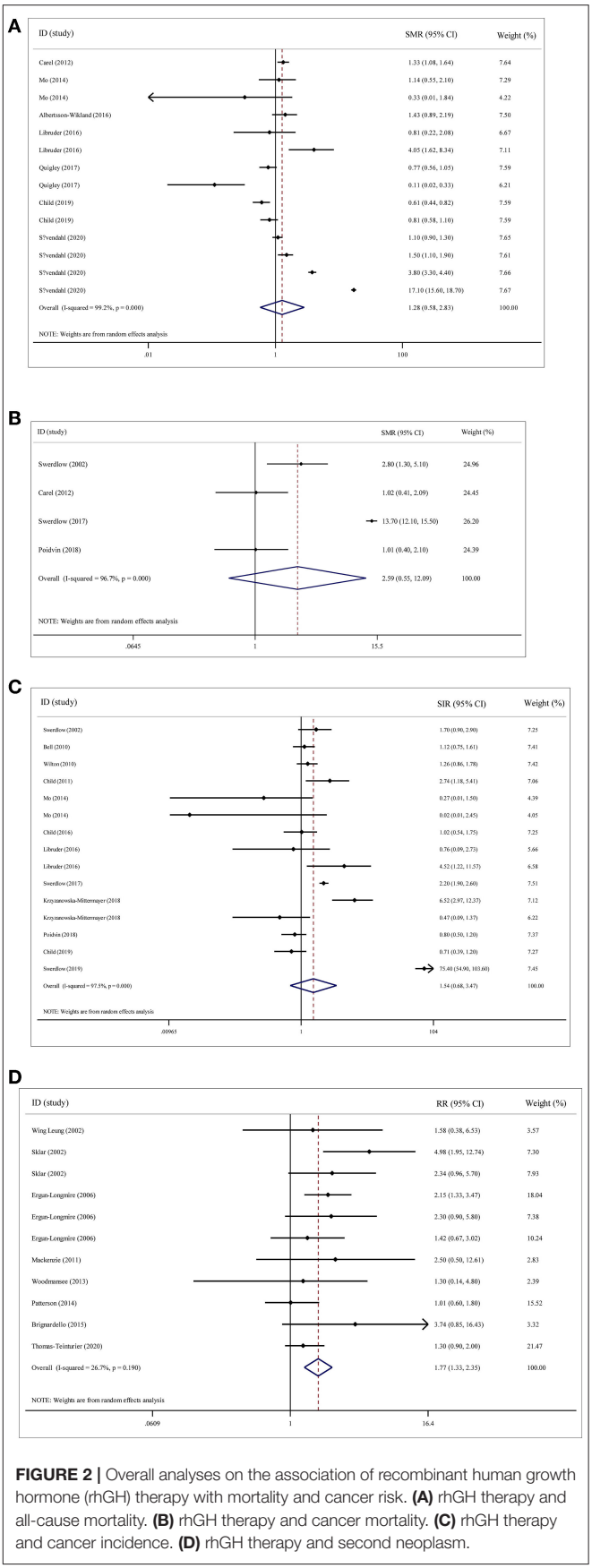


FIGURE 2 | Overall analyses on the association of recombinant human growth hormone (rhGH) therapy with mortality and cancer risk. **(A)** rhGH therapy and all-cause mortality. **(B)** rhGH therapy and cancer mortality. **(C)** rhGH therapy and cancer incidence. **(D)** rhGH therapy and second neoplasm.

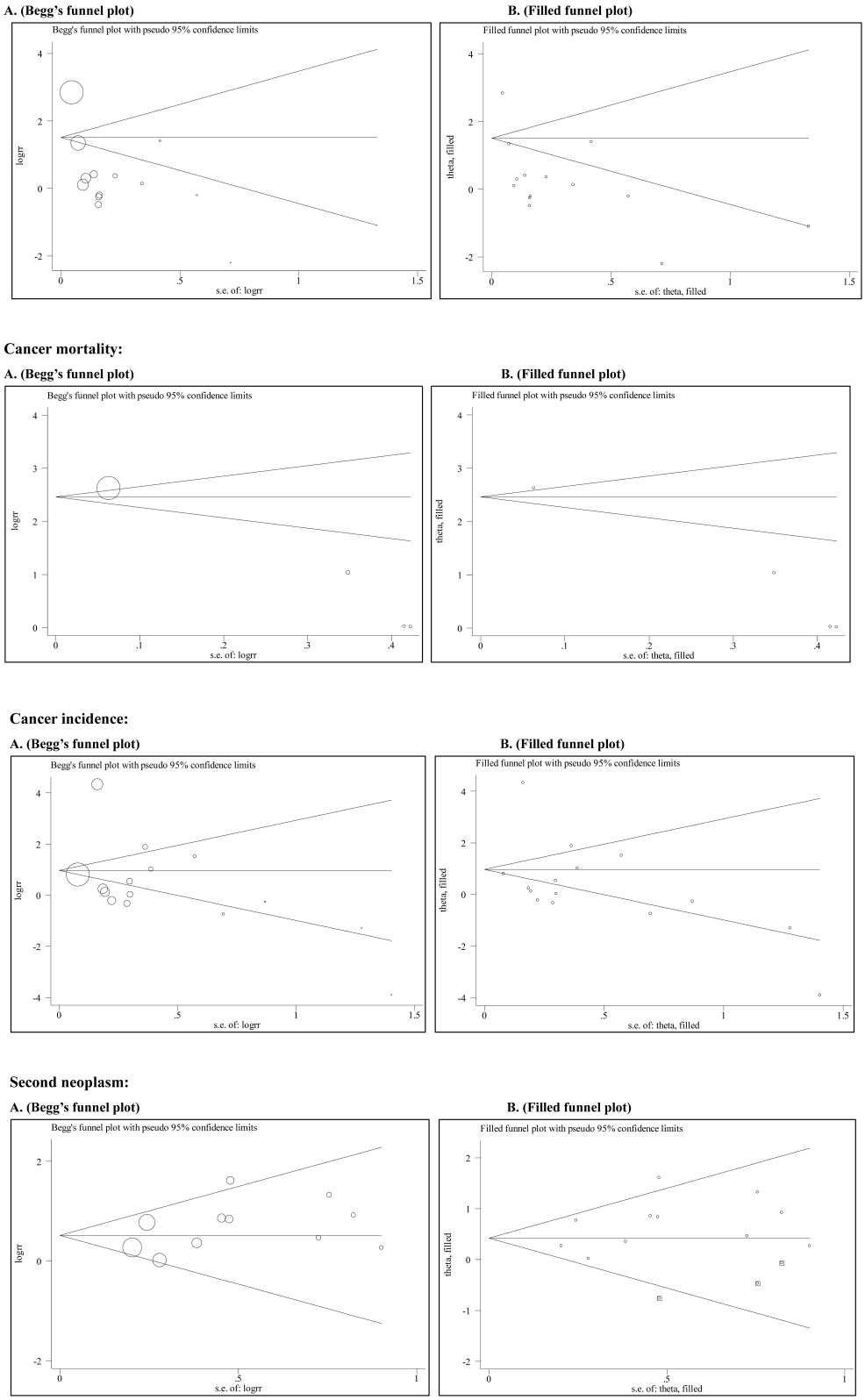


FIGURE 3 | The Begg's and filled funnel plots for the association of rhGH therapy with mortality and cancer risk. All-cause mortality: **(A)** Begg's funnel plot, **(B)** Filled funnel plot. Cancer mortality: **(A)** Begg's funnel plot, **(B)** Filled funnel plot. Cancer incidence: **(A)** Begg's funnel plot, **(B)** Filled funnel plot. Second neoplasm: **(A)** Begg's funnel plot, **(B)** Filled funnel plot.

of second neoplasm ($P = 0.167$). Further investigations using the "trim and fill" method showed that 3 theoretically missing studies were required to make Begg's funnel plot symmetrical for the occurrence of the second neoplasm. However, no study was required in theory for standardized incidence of cancer.

There was statistical evidence of asymmetry by using Eggers's tests in all-cause mortality ($P = 0.015$) and cancer mortality ($P = 0.008$). The "trim and fill" method did not produce any derivations from the original estimates.

Subgroup Analyses

A series of prespecified subgroup analyses were conducted to account for possible sources of between-study heterogeneity for rhGH therapy with the all-cause mortality, cancer mortality, the standardized incidence of cancer, and the occurrence of second neoplasm (**Table 1**).

By geographic regions based on the all-cause SMR, the association between pediatric somatropin treatment and the all-cause mortality was not statistically significant in Europe (SMR = 1.92, 95% CI: 0.71–5.23, $P = 0.202$; $I^2 = 99.4\%$; $Tau^2 = 1.938$) and Asia (SMR = 1.90, 95% CI: 0.39–9.17, $P = 0.424$; $I^2 = 80.6\%$; $Tau^2 = 1.044$) and also no significance was detected between rhGH therapy and cancer mortality in children in Europe (SMR = 1.47, 95% CI: 0.73–2.96, $P = 0.279$; $I^2 = 59.3\%$; $Tau^2 = 0.227$) based on the geographical areas of cancer SMR. Based on cancer SIR by geographic regions, the association was nonsignificant between rhGH therapy and standard cancer incidence in both Europe (SIR = 1.09, 95% CI: 0.08–14.50, $P = 0.951$; $I^2 = 98.8\%$; $Tau^2 = 8.071$) and Asia (SIR = 2.09, 95% CI: 0.37–11.81, $P = 0.058$; $I^2 = 65.8\%$; $Tau^2 = 1.046$). The association between childhood rhGH therapy and second neoplasm was statistically significant in North America (RR = 2.20, 95% CI: 1.61–3.02, $P < 0.001$; $I^2 = 0.00\%$; $Tau^2 = 0.000$). However, the statistical significance was not demonstrated in Europe (RR = 1.57, 95% CI: 0.93–2.66, $P = 0.094$; $I^2 = 12.9\%$; $Tau^2 = 0.051$).

By gender based on the all-cause SMR, the association between rhGH therapy and all-cause mortality was not statistically significant in either boys (SMR = 2.50, 95% CI: 0.81–7.69, $P = 0.110$; $I^2 = 99.4\%$; $Tau^2 = 1.629$) or girls (SMR = 3.01, 95% CI: 0.71–12.78, $P = 0.135$; $I^2 = 99.2\%$; $Tau^2 = 2.663$).

By study design based on the all-cause SMR, the association between rhGH therapy and all-cause mortality in children was not statistically significant in prospective cohorts (SMR = 1.16, 95% CI: 0.44–3.10, $P = 0.765$; $I^2 = 99.4\%$; $Tau^2 = 1.629$) and in retrospective cohorts, the SMR for rhGH therapy and all-cause mortality was 1.56 (95% CI: 1.02–2.38, $P = 0.041$; $I^2 = 60.6\%$; $Tau^2 = 0.102$). Based on the study type of cancer SMR, there was no statistical significance between growth hormone therapy and tumor mortality in retrospective cohorts (SMR = 1.47, 95% CI: 0.73–2.96, $P = 0.279$; $I^2 = 59.3\%$; $Tau^2 = 0.227$). Based on the study design of cancer SIR, in prospective cohorts, there was no statistical significance between rhGH therapy and the standard incidence of tumor (SIR =1.22, 95% CI: 0.15–10.31, $P = 0.853$; $I^2 = 99.1\%$; $Tau^2 = 5.286$), yet significance was attained in retrospective cohorts (SIR = 1.53, 95% CI: 1.02–2.31, $P = 0.040$; $I^2 = 75.8\%$; $Tau^2 = 0.286$). By the study design based on the second neoplasm, the association between rhGH therapy and second neoplasm reached statistical significance (RR = 1.77, 95% CI: 1.33–2.35, $P < 0.001$; $I^2 = 26.7\%$; $Tau^2 = 0.055$).

By risk based on all-cause SMR, in children with low risk (SMR = 1.25, 95% CI: 1.17–1.34, $P < 0.001$; $I^2 = 14.4\%$; $Tau^2 = 0.009$), moderate risk (SMR = 4.00, 95% CI: 3.50–4.57, $P < 0.001$; $I^2 = 69.0\%$; $Tau^2 = 0.061$), or high risk (SMR = 16.88, 95% CI: 14.52–19.63, $P < 0.001$; $I^2 = 0.1\%$; $Tau^2 = 0.101$), the relationship between rhGH therapy and all-cause mortality was statistically significant. Based on the risk of cancer SMR, there was statistical significance between rhGH therapy and cancer mortality in children with not-high risk (SMR = 8.28, 95% CI: 1.62–42.41, $P = 0.011$; $I^2 = 99.6\%$; $Tau^2 = 2.714$). In addition, rhGH therapy did not significantly affect standard tumor incidence among children at not-high risk based on the risk of cancer SIR (SIR = 1.88, 95% CI: 0.99–3.57, $P = 0.055$; $I^2 = 96.9\%$; $Tau^2 = 0.602$).

By duration of rhGH therapy based on the all-cause SMR, the association between rhGH therapy and all-cause mortality was not statistically significant when treatment duration was ≥5 years (SMR = 1.96, 95% CI: 0.83–4.65, $P = 0.126$; $I^2 = 95.8\%$; $Tau^2 = 1.427$). However, when the treatment time was <5 years (SMR =3.20, 95% CI: 1.78–5.76, $P < 0.001$; $I^2 = 98.2\%$; $Tau^2 = 1.665$), the association was significant.

By overall rhGH exposure dose based on all-cause SMR, the association between rhGH therapy and all-cause mortality was not statistically significant when rhGH exposure during childhood was <25 mg/kg (SMR = 2.03, 95% CI: 0.62–6.59, $P = 0.241$; $I^2 = 98.8\%$; $Tau^2 = 2.493$), 25–50 mg/kg (SMR = 2.85, 95% CI: 0.89–9.09, $P = 0.077$; $I^2 = 98.3\%$; $Tau^2 = 1.711$), and 50–100 mg/kg (SMR = 2.64, 95% CI: 0.81–8.55, $P = 0.106$; $I^2 = 96.9\%$; $Tau^2 = 1.341$), whereas the association was statistically significant when total rhGH exposure was ≥100 mg/kg (SMR = 3.32, 95% CI: 1.22–9.08, $P = 0.019$; $I^2 = 85.8\%$; $Tau^2 = 0.832$).

By follow-up period based on all-cause SMR, there was no statistically significant association between rhGH therapy and all-cause mortality when the follow-up period ≥10 years (SMR = 0.98, 95% CI: 0.75–1.29, $P = 0.899$; $I^2 = 78.3\%$; $Tau^2 = 0.127$). The association between rhGH therapy and all-cause mortality was statistically significant in studies with follow-up duration <10 years (SMR = 4.62, 95% CI: 1.19–18.01, $P = 0.027$; $I^2 = 99.6\%$; $Tau^2 = 1.435$). The association between rhGH therapy and cancer mortality was not statistically significant in studies with follow-up duration ≥10 years (SMR = 2.59, 95% CI: 0.55–12.09, $P = 0.228$; $I^2 = 96.7\%$; $Tau^2 = 2.361$) based on cancer SMR. Based on cancer SIR of follow-up, there was no statistically significant association between rhGH therapy and standard cancer incidence at follow-up times ≥10 years (SIR =1.54, 95% CI: 0.68–3.47, $P < 0.001$; $I^2 = 97.5\%$; $Tau^2 = 2.287$). Nevertheless, there was a statistically significant relationship between second neoplasm and rhGH therapy (RR = 1.77, 95% CI: 1.33–2.35, $P < 0.001$; $I^2 = 26.7\%$; $Tau^2 = 0.055$).

DISCUSSION

To the best of our knowledge, this is, thus far the most comprehensive meta-analysis that has examined the association

TABLE 1 | Overall and subgroup analyses on the association of recombinant human growth hormone therapy with mortality and cancer risk.

Group	Number of qualified studies	Mortality or cancer risk		Tau^2
		RR (95% CI); *P*	I^2	
Overall analyses				
All cause SMR	14	1.28 (0.58–2.84); 0.547	99.2%	2.154
Cancer SMR	4	2.59 (0.55–12.09); 0.228	96.7%	2.361
Cancer SIR	15	1.54 (0.68–3.47); 0.229	97.5%	2.287
RR SN	11	1.77 (1.33–2.35); 0.001	26.7%	0.055
Subgroup analyses based on mortality or cancer risk				
By region based on All cause SMR				
Europe	8	1.92 (0.71–5.23); 0.202	99.4%	1.938
Asia	2	1.90 (0.39–9.17); 0.424	80.6%	1.044
International	4	0.66 (0.47–0.92); 0.016	65.1%	0.070
By region based on Cancer SMR				
Europe	3	1.47 (0.73–2.96); 0.279	59.3%	0.227
By region based on Cancer SIR				
Europe	5	1.09 (0.08–14.50); 0.951	98.8%	8.071
Asia	7	2.09 (0.37–11.81); 0.058	65.8%	1.046
International	2	1.59 (0.98–2.57); 0.404	85.3%	0.314
By region based on RR SN				
North America	6	2.20 (1.61–3.02); <0.001	0.0%	0.000
Europe	3	1.57 (0.93–2.66); 0.094	12.9%	0.051
International	2	1.03 (0.61–1.75); 0.904	0.0%	0.000
By gender based on All cause SMR				
Boys	5	2.50 (0.81–7.69); 0.110	99.4%	1.629
Girls	5	3.01 (0.71–12.78); 0.135	99.2%	2.663
By study design based on All cause SMR				
Prospective	10	1.16 (0.44–3.10); 0.765	99.4%	2.334
Retrospective	4	1.56 (1.02–2.38); 0.041	60.6%	0.102
By study design based on Cancer SMR				
Retrospective	3	1.47 (0.73–2.96); 0.279	59.3%	0.227
By study design based on Cancer SIR				
Prospective	5	1.22 (0.15–10.31); 0.853	99.1%	5.286
Retrospective	10	1.53 (1.02–2.31); 0.040	75.8%	0.286
By study design based on RR SN				
Retrospective	11	1.77 (1.33–2.35); <0.001	26.7%	0.055
By risk based on All cause SMR				
Low	54	1.25 (1.17–1.34); <0.001	14.4%	0.009
Moderate	22	4.00 (3.50–4.57); <0.001	69.0%	0.061
High	21	16.88 (14.52–19.63); <0.001	90.1%	0.101
By risk based on Cancer SMR				
Not high	4	8.28 (1.62–42.41); 0.011	99.6%	2.714
By risk based on Cancer SIR				
Not high	6	1.88 (0.99–3.57); 0.055	96.9%	0.602
By GH treatment duration (years) based on All–cause SMR				
<5	19	3.20 (1.78–5.76); <0.001	98.2%	1.665
≥5	8	1.96 (0.83–4.65); 0.126	95.8%	1.427
By overall GH exposure dose (mg/kg) based on All cause–SMR				
<25	7	2.03 (0.62–6.59); 0.241	98.8%	2.493
25–50	5	2.85 (0.89–9.09); 0.077	98.3%	1.711
50–100	4	2.64 (0.81–8.55); 0.106	96.9%	1.341
≥100	4	3.32 (1.22–9.08); 0.019	85.8%	0.832

(Continued)

TABLE 1 | Continued

Group	Number of qualified studies	Mortality or cancer risk		Tau^2
		RR (95% CI); *P*	I^2	
By follow up (years) based on All cause-SMR				
≥10	11	0.98 (0.75–1.29); 0.899	78.3%	0.127
<10	3	4.62 (1.19–18.01); 0.027	99.6%	1.435
By follow up (years) based on Cancer SMR				
≥10	4	2.59 (0.55–12.09); 0.228	96.7%	2.361
By follow up (years) based on Cancer SIR				
≥10	15	1.54 (0.68–3.47); 0.299	97.5%	2.287
By follow up (years) based on RR SN				
≥10	11	1.77 (1.33–2.35); <0.001	26.7%	0.055

RR, risk ratio; 95% CI, 95% confidence interval, SMR, standardized mortality ratios; SIR, standardized incidence ratio; SN, second neoplasm; GH, growth hormone therapy.

between rhGH therapy during childhood and multiple clinical outcomes including the all-cause mortality, cancer mortality, standard cancer incidence, and second neoplasm. Our key findings suggested that rhGH therapy in childhood had no deleterious effects on all-cause mortality, cancer mortality, and standard cancer incidence. In contrast, rhGH therapy was a risk factor for the development of the second neoplasm. Furthermore, our analyses suggested that differences in the geographic region, gender, treatment duration, mean rhGH dose, overall rhGH exposure dose, and initial disease accounted for heterogeneity. Our findings highlight the relative safety of growth hormone use in childhood and provide high-quality evidence for pediatrics, particularly for these conditions requiring rhGH therapy.

Extending the findings of previous individual studies that assessed only one or two clinical outcomes after rhGH therapy, we, in this meta-analysis, comprehensively evaluated all the possible outcomes in both the overall analyses and subgroup analyses. It is worth noting that all-cause mortality and cancer incidence were significantly higher than expected in the low- and intermediate-risk groups. Although only 2 articles were involved in the analysis of total exposure to rhGH in children, the all-cause mortality rate was significantly higher than expected when the total exposure dose was over 100 mg/kg based on the results of the analysis of the overall exposure dose. However, this dose needs to be determined by future studies. Moreover, we also interestingly noticed that all-cause mortality was significantly lower than expected for both boys and girls. Although the exact mechanisms behind these positive findings are not fully understood, we agree that further well-designed, long-term studies are warranted to further enrich our understanding of the clinical implication of rhGH therapy in childhood in future mortality risk in adulthood.

The current meta-analysis is based on the previous meta-analysis conducted by Deodati et al. (12) by pooling the results of 12 studies, who found no significant increase in the malignant tumor SMRs, yet overall cancer SIRs (2.74; 95% CI: 1.18–4.41) and RRs of second tumors (1.99; 95% CI: 1.28–3.08) were significantly increased. In the present meta-analysis, by contrast, we found that all-cause mortality and malignancy incidence were significantly lower than expected, that is, rhGH therapy was not a risk factor for all-cause mortality and malignancy incidence. The reasons for the conflicting observations between the meta-analysis by Deodati et al. (12) and this meta-analysis are mainly because of the power to detect statistical significance, as we incorporated the results from 24 articles.

Our finding that no significant association was found between the dose of rhGH and mortality and cancer incidence makes causality less likely. However, some studies have reported an increased incidence of bone cancer and bladder cancer in patients treated with rhGH and in patients with Hodgkin lymphoma (2, 25). Nonetheless in this meta-analysis, we did not conduct relevant subgroup analysis due to a lack of data on the initial disease of patients with detailed types of cancers. In addition, we believe that rhGH therapy should be carried out with caution in high-risk patients and that the start of rhGH therapy should be carefully discussed (34).

Available evidence suggests an increased risk of secondary tumors in rhGH recipients. Growth hormone is potent mitosis and anti-apoptotic hormone, and increased activity of the growth hormone/IGF-I axis is associated with an increased risk of cancer (35). Therefore, with the use of growth hormone therapy, the researchers' vigilance against the potential cancer risk accompanied this treatment from the beginning. Animal experiments showed that in spontaneous pygmy rats lacking rhGH, the administration of the carcinogen N-methyl-N-nitrosourea did not induce breast tumors. However, the tumors were developed in GH-treated rats (36). Moreover, after stopping hormone replacement, almost all the tumors have completely degenerated in animal models of rhGH receptor knockout mice hybridizing with Tag mice prone to prostate tumors, and similar findings were described by other investigators (35, 37). High IGF-1 or high growth hormone levels may induce messenger RNA alterations or other molecular changes and angiogenesis and inhibit apoptosis. This may further stimulate the carcinogenic potential that already exists (33, 38, 39). Molecular signaling pathways that affect cell proliferation, differentiation, and survival are regulated by the GH-IGF-1 axis. The carcinogenic process interacts with GH-IGF-1 signaling pathways, employs these physiological signaling

pathways, and converts them into abnormal signaling pathways (33, 38).

Generally, the findings of this meta-analysis are reassuring, but some biases, confounding factors, and weaknesses limit the value and interpretation of all data reported to date. Detailed information on dosage, duration of treatment, and primary disease in children need more literature support and although the relevant subgroup analysis was conducted in this study, the number of relevant articles was relatively small. Future prospective studies are also needed to confirm these results and answer more difficult questions about the appropriate period to start GH therapy after achieving complete remission, and how to deal with children with "chronic" low-grade tumor diseases and growth hormone deficiency (GHD). In addition, more research is required on the optimal dosage of rhGH therapy (34).

CONCLUSION

Our findings indicate that rhGH therapy is not related to all-cause mortality, cancer mortality, and cancer incidence, yet it seems to trigger a second tumor risk. The long-term safety of growth hormone therapy still deserves more attention as mortality from certain causes is increasing, and the need for long-term monitoring remains essential.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JH, ZZ, and WN: conceived and designed the experiments. MH, XW, and ZZ: performed the experiments. MH, XD, and JH: analyzed the data and contributed materials/analysis tools. MH, XW, JH, and WN: wrote the article. All the authors read and approved the final manuscript before submission.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.866295/full#supplementary-material

REFERENCES

1. Swerdlow AJ, Cooke R, Beckers D, Butler G, Carel JC, Cianfarani S, et al. Risk of meningioma in European patients treated with growth hormone in childhood: results from the SAGhE cohort. *J Clin Endocrinol Metab.* (2019) 104:658–64. doi: 10.1210/jc.2018-01133
2. Child CJ, Zimmermann AG, Chrousos GP, Cummings E, Deal CL, Hasegawa T, et al. Safety outcomes during pediatric GH therapy: final results from the prospective GeNeSIS observational program. *J Clin Endocrinol Metab.* (2019) 104:379–89. doi: 10.1210/jc.2018-01189
3. Tidblad A, Bottai M, Kieler H, Albertsson-Wikland K, Sävendahl L. Association of childhood growth hormone treatment with long-term cardiovascular morbidity. *JAMA Pediatr.* (2021) 175:e205199. doi: 10.1001/jamapediatrics.2020.5199
4. Clayton PE, Cowell CT. Safety issues in children and adolescents during growth hormone therapy–a review. *Growth Horm IGF Res.* (2000) 10:306–17. doi: 10.1054/ghir.2000.0175
5. Bell J, Parker KL, Swinford RD, Hoffman AR, Maneatis T, Lippe B. Long-term safety of recombinant human growth hormone in children. *J Clin Endocrinol Metab.* (2010) 95:167–77. doi: 10.1210/jc.2009-0178
6. Allen DB, Backeljauw P, Bidlingmaier M, Biller BM, Boguszewski M, Burman P, et al. GH safety workshop position paper: a critical appraisal of recombinant human GH therapy in children and adults. *Eur J Endocrinol.* (2016) 174:P1–9. doi: 10.1530/EJE-15-0873
7. (1988). Leukaemia in patients treated with growth hormone. *Lancet.* 1:1159–60. doi: 10.1016/S0140-6736(88)91968-X
8. Allen DB, Rundle AC, Graves DA, Blethen SL. Risk of leukemia in children treated with human growth hormone: review and reanalysis. *J Pediatr.* (1997) 131:S32–36. doi: 10.1016/S0022-3476(97)70008-8
9. Sklar CA, Mertens AC, Mitby P, Occhiogrosso G, Qin J, Heller G, et al. Risk of disease recurrence and second neoplasms in survivors of childhood cancer treated with growth hormone: a report from the Childhood Cancer Survivor Study. *J Clin Endocrinol Metab.* (2002) 87:3136–41. doi: 10.1210/jcem.87.7.8606
10. Ergun-Longmire B, Mertens AC, Mitby P, Qin J, Heller G, Shi W, et al. Growth hormone treatment and risk of second neoplasms in the childhood cancer survivor. *J Clin Endocrinol Metab.* (2006) 91:3494–8. doi: 10.1210/jc.2006-0656
11. Raman S, Grimberg A, Waguespack SG, Miller BS, Sklar CA, Meacham LR, et al. Risk of neoplasia in pediatric patients receiving growth hormone therapy—a report from the pediatric endocrine society drug and therapeutics committee. *J Clin Endocrinol Metab.* (2015) 100:2192–203. doi: 10.1210/jc.2015-1002
12. Deodati A, Ferroli BB, Cianfarani S. Association between growth hormone therapy and mortality, cancer and cardiovascular risk: systematic review and meta-analysis. *Growth Horm IGF Res.* (2014) 24:105–11. doi: 10.1016/j.ghir.2014.02.001
13. Sävendahl L, Cooke R, Tidblad A, Beckers D, Butler G, Cianfarani S, et al. Long-term mortality after childhood growth hormone treatment: the SAGhE cohort study. *Lancet Diabetes Endocrinol.* (2020) 8:683–92. doi: 10.1016/S2213-8587(20)30163-7
14. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* (2021) 372:n71. doi: 10.1136/bmj.n71
15. Altman DG, Bland JM. Interaction revisited: the difference between two estimates. *BMJ.* (2003) 326:219. doi: 10.1136/bmj.326.7382.219
16. Carel JC, Ecosse E, Landier F, Meguellati-Hakkas D, Kaguelidou F, Rey G, et al. Long-term mortality after recombinant growth hormone treatment for isolated growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study. *J Clin Endocrinol Metab.* (2012) 97:416–25. doi: 10.1210/jc.2011-1995
17. Mo D, Hardin DS, Erfurth EM, Melmed S. Adult mortality or morbidity is not increased in childhood-onset growth hormone deficient patients who received pediatric GH treatment: an analysis of the Hypopituitary Control and Complications Study (HypoCCS). *Pituitary.* (2014) 17:477–85. doi: 10.1007/s11102-013-0529-6
18. Albertsson-Wikland K, Mårtensson A, Sävendahl L, Niklasson A, Bang P, Dahlgren J, et al. Mortality is not increased in recombinant human growth hormone-treated patients when adjusting for birth characteristics. *J Clin Endocrinol Metab.* (2016) 101:2149–59. doi: 10.1210/jc.2015-3951
19. Libruder C, Blumenfeld O, Dichtiar R, Laron Z, Zadik Z, Shohat T, et al. Mortality and cancer incidence among patients treated with recombinant growth hormone during childhood in Israel. *Clin Endocrinol (Oxf).* (2016) 85:813–8. doi: 10.1111/cen.13131
20. Quigley CA, Child CJ, Zimmermann AG, Rosenfeld RG, Robison LL, Blum WF. Mortality in children receiving growth hormone treatment of

growth disorders: data from the genetics and neuroendocrinology of short stature international study. *J Clin Endocrinol Metab.* (2017) 102:3195–205. doi: 10.1210/jc.2017-00214
21. Swerdlow AJ, Higgins CD, Adlard P, Preece MA. Risk of cancer in patients treated with human pituitary growth hormone in the UK, 1959-85: a cohort study. *Lancet.* (2002) 360:273–7. doi: 10.1016/S0140-6736(02)09519-3
22. Wilton P, Mattsson AF, Darendeliler F. Growth hormone treatment in children is not associated with an increase in the incidence of cancer: experience from KIGS (Pfizer International Growth Database). *J Pediatr.* (2010) 157:265–70. doi: 10.1016/j.jpeds.2010.02.028
23. Child CJ, Zimmermann AG, Woodmansee WW, Green DM, Li JJ, Jung H, et al. Assessment of primary cancers in GH-treated adult hypopituitary patients: an analysis from the Hypopituitary Control and Complications Study. *Eur J Endocrinol.* (2011) 165:217–23. doi: 10.1530/EJE-11-0286
24. Child CJ, Zimmermann AG, Jia N, Robison LL, Brämswig JH, Blum WF. Assessment of primary cancer incidence in growth hormone-treated children: comparison of a multinational prospective observational study with population databases. *Horm Res Paediatr.* (2016) 85:198–206. doi: 10.1159/000444124
25. Swerdlow AJ, Cooke R, Beckers D, Borgstrom B, Butler G, Carel JC, et al. Cancer risks in patients treated with growth hormone in childhood: the SAGhE European Cohort Study. *J Clin Endocrinol Metab.* (2017) 102:1661–72. doi: 10.1210/jc.2016-2046
26. Krzyzanowska-Mittermayer K, Mattsson AF, Maiter D, Feldt-Rasmussen U, Camacho-Hübner C, Luger A, et al. New neoplasm during GH replacement in adults with pituitary deficiency following malignancy: a KIMS analysis. *J Clin Endocrinol Metab.* (2018) 103:523–31. doi: 10.1210/jc.2017-01899
27. Poidvin A, Carel JC, Ecosse E, Levy D, Michon J, Coste J. Increased risk of bone tumors after growth hormone treatment in childhood: a population-based cohort study in France. *Cancer Med.* (2018) 7:3465–73. doi: 10.1002/cam4.1602
28. Leung W, Rose SR, Zhou Y, Hancock ML, Burstein S, Schriock EA, et al. Outcomes of growth hormone replacement therapy in survivors of childhood acute lymphoblastic leukemia. *J Clin Oncol.* (2002) 20:2959–64. doi: 10.1200/JCO.2002.09.142
29. Mackenzie S, Craven T, Gattamaneni HR, Swindell R, Shalet SM, Brabant G. Long-term safety of growth hormone replacement after CNS irradiation. *J Clin Endocrinol Metab.* (2011) 96:2756–61. doi: 10.1210/jc.2011-0112
30. Woodmansee WW, Zimmermann AG, Child CJ, Rong Q, Erfurth EM, Beck-Peccoz P, et al. Incidence of second neoplasm in childhood cancer survivors treated with GH: an analysis of GeNeSIS and HypoCCS. *Eur J Endocrinol.* (2013) 168:565–73. doi: 10.1530/EJE-12-0967
31. Patterson BC, Chen Y, Sklar CA, Neglia J, Yasui Y, Mertens A, et al. Growth hormone exposure as a risk factor for the development of subsequent neoplasms of the central nervous system: a report from the childhood cancer survivor study. *J Clin Endocrinol Metab.* (2014) 99:2030–7. doi: 10.1210/jc.2013-4159
32. Brignardello E, Felicetti F, Castiglione A, Fortunati N, Matarazzo P, Biasin E, et al. GH replacement therapy and second neoplasms in adult survivors of childhood cancer: a retrospective study from a single institution. *J Endocrinol Invest.* (2015) 38:171–6. doi: 10.1007/s40618-014-0179-1
33. Thomas-Teinturier C, Oliver-Petit I, Pacquement H, Fresneau B, Allodji RS, Veres C, et al. Influence of growth hormone therapy on the occurrence of a second neoplasm in survivors of childhood cancer. *Eur J Endocrinol.* (2020) 183:471–80. doi: 10.1530/EJE-20-0369
34. van Santen HM. Safety of GH after treatment for childhood cancer. *Eur J Endocrinol.* (2020) 183:C15–c18. doi: 10.1530/EJE-20-0965
35. Tidblad A. The history, physiology and treatment safety of growth hormone. *Acta Paediatr.* (2021) 111:215–24 doi: 10.1111/apa.15948
36. Shen Q, Lantvit DD, Lin Q, Li Y, Christov K, Wang Z, et al. Advanced rat mammary cancers are growth hormone dependent. *Endocrinology.* (2007) 148:4536–44. doi: 10.1210/en.2007-0513
37. Wang Z, Prins GS, Coschigano KT, Kopchick JJ, Green JE, Ray VH, et al. Disruption of growth hormone signaling retards early stages of prostate carcinogenesis in the C3(1)/T antigen mouse. *Endocrinology.* (2005) 146:5188–96. doi: 10.1210/en.2005-0607
38. Brunet-Dunand SE, Vouyovitch C, Araneda S, Pandey V, Vidal LJ, Print C, et al. Autocrine human growth hormone promotes tumor angiogenesis in mammary carcinoma. *Endocrinology.* (2009) 150:1341–52. doi: 10.1210/en.2008-0608
39. Sustarsic EG, Junnila RK, Kopchick JJ. Human metastatic melanoma cell lines express high levels of growth hormone receptor and respond to GH treatment. *Biochem Biophys Res Commun.* (2013) 441:144–50. doi: 10.1016/j.bbrc.2013.10.023

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Endocrinology

ORIGINAL RESEARCH
published: 29 April 2022
doi: 10.3389/fendo.2022.864908

A Randomized Controlled Phase 3 Study on the Efficacy and Safety of Recombinant Human Growth Hormone in Children With Idiopathic Short Stature

Jinna Yuan[1], Junfen Fu[1], Haiyan Wei[2], Gaixiu Zhang[3], Yanfeng Xiao[4], Hongwei Du[5], Wei Gu[6], Yanhong Li[7], Linqi Chen[8], Feihong Luo[9], Yan Zhong[10] and Haihong Gong[11]*

[1] Endocrinology Department, Children's Hospital of Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou, China, [2] Department of Endocrinology, Genetics and Metabolism, Zhengzhou Children's Hospital, Zhengzhou, China, [3] Department of Pediatrics and Endocrinology, Children's Hospital of Shanxi, Taiyuan, China, [4] Department of Pediatrics, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China, [5] Department of Pediatrics and Endocrinology, The First Hospital of Jilin University, Jilin, China, [6] Department of Endocrinology, Nanjing Children's Hospital, Nanjing, China, [7] Department of Pediatrics, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [8] Department of Endocrinology, Genetics and Metabolism, Children's Hospital of Soochow University, Suzhou, China, [9] Department of Endocrinology, Children's Hospital of Fudan University, Shanghai, China, [10] Children Health Division, Hunan Children's Hospital, Changsha, China, [11] Department of Pediatrics, Jiangsu Provincial People's Hospital, NanjingChina

OPEN ACCESS

Edited by:
Brenda Kohn,
NYU Grossman School of Medicine,
United States

Reviewed by:
George Arthur Werther,
Royal Children's Hospital, Australia
Yu Yang,
Jiangxi Provincial Children's Hospital,
China

***Correspondence:**
Junfen Fu
fjf68@zju.edu.cn

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 29 January 2022
Accepted: 09 March 2022
Published: 29 April 2022

Citation:
Yuan J, Fu J, Wei H, Zhang G, Xiao Y, Du H, Gu W, Li Y, Chen L, Luo F, Zhong Y and Gong H (2022) A Randomized Controlled Phase 3 Study on the Efficacy and Safety of Recombinant Human Growth Hormone in Children With Idiopathic Short Stature. Front. Endocrinol. 13:864908. doi: 10.3389/fendo.2022.864908

Background: To evaluate the safety and efficacy of daily somatropin (Jintropin®), a recombinant human growth hormone, in prepubertal children with ISS in China.

Methods: This study was a multicenter, randomized, controlled, open-label, phase 3 study. All subjects were randomized 3:1 to daily somatropin 0.05 mg/kg/day or no treatment for 52 weeks. A total of 481 subjects with a mean baseline age of 5.8 years were enrolled in the study. The primary endpoint was change in (△) height standard deviation score (HT-SDS) for chronological age (CA). Secondary endpoints included △height from baseline; △bone age (BA)/CA; △height velocity (HV) and △insulin-like growth factor 1 (IGF-1 SDS).

Results: △HT-SDS at week 52 was 1.04 ± 0.31 in the treatment group and 0.20 ± 0.33 in the control group ($P < 0.001$). At week 52, statistical significance was observed in the treatment group compared with control for △height (10.19 ± 1.47 cm vs. 5.85 ± 1.80 cm; $P < 0.001$), △BA/CA (0.04 ± 0.09 vs. 0.004 ± 0.01; $P < 0.001$), △HV (5.17 ± 3.70 cm/year vs. 0.75 ± 4.34 cm/year; $P < 0.001$), and △IGF-1 SDS (2.31 ± 1.20 vs. 0.22 ± 0.98; $P < 0.001$). The frequencies of treatment-emergent adverse events (TEAEs) were similar for the treatment and the control groups (89.8% vs. 82.4%); most TEAEs were mild to moderate in severity and 23 AEs were considered study-drug related.

Conclusions: Daily subcutaneous administration of somatropin at 0.05 mg/kg/day for 52 weeks demonstrated improvement in growth outcomes and was well tolerated with a favorable safety profile.

Trial Registration: ClinicalTrials.gov (identifier: NCT03635580). URL: https://clinicaltrials.gov/ct2/show/NCT03635580

Keywords: efficacy, safety, rhGH, idiopathic short stature, China

1 INTRODUCTION

Idiopathic short stature (ISS) refers to a condition characterized by a height more than 2 standard deviation score (SDS) below the corresponding mean height for a given age, gender, and population that has no evidence of underlying pathology (1). ISS accounts for 80% of children with short stature of a height below –2 SDS (1). In 2 retrospective, single-center studies, ISS was found in approximately 40% of the study population (2, 3).

The use of growth hormone (GH) was approved by the U.S. Food and Drug Administration (FDA) in 2003 for children with ISS with a height of more than 2.25 SDS below the mean height and who are unlikely to attain normal adult height (4). A consensus statement published by the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology recommended that children with ISS at a height of less than –2 SDS and were also more than 2 SDS below midparental height could be treated with GH (5).

The cause of ISS remains unknown and children with ISS have normal birth weight and GH levels. It is postulated that it is due to genetic aberrations along the GH-insulin-like growth factor 1 (IGF-1) pathway and in the short stature homeobox-containing (SHOX) gene (4). The purpose of treatment is to enable individuals with ISS to attain normal, or close to normal, adult height and avoid any psychological issues that come with extreme or unacceptable short stature. However, individual responses to GH are highly variable; treatment is considered successful if, in the first year, a change in ($\triangle$) height SDS (HT-SDS) of more than 0.3–0.5 and an increment in height velocity (HV) of more than 3 cm/year is achieved (5).

Longer-term treatment with GH has been reported to increase mean adult height by 3.5–7.5 cm in children with ISS and had a safety profile similar to outcomes in other GH disorders; most adverse events (AEs) were mild in severity with a low risk of high-grade toxicities (5). In China, there were several studies demonstrating the clinical benefit of recombinant human GH (rhGH) therapy in children with ISS compared with baseline (6, 7). However, most of these studies were retrospective and observational by design. There is a lack of clear data on the effectiveness and safety of rhGH therapy in children with ISS in China.

Somatropin (Jintropin®, GeneScience Pharmaceuticals, Changchun, China) is a daily rhGH therapy that was approved by the China FDA in 2005 for the treatment of GH deficiency, severe burns, Noonan syndrome, short stature caused by SHOX deficiency, achondroplasia, gonad hypoplasia (Turner syndrome), children small for gestational age (failure to catch-up growth at age 2 years), hypothalamic-pituitary disorder caused by GH deficiency, and short bowel syndrome in patients receiving specialized nutritional support. Somatropin has demonstrated safety and efficacy in all the approved indications.

We conducted a phase 3 study to evaluate the safety and efficacy of daily somatropin in prepubertal children with ISS in China.

2 METHODS

2.1 Subjects

Inclusion criteria were: 1) aged between 4–9 years in girls and 4–10 years in boys; 2) HT-SDS ≤–2.25 SD of the average height of normal children of the same age and gender based on the Chinese general population at the time of screening (8); 3) peaked stimulated GH ≥10 ng/mL; 4) bone age (BA) ≤ actual age + 6 months; 5) prepubertal (Tanner stage 1); and 6) no previous history of GH treatment.

Exclusion criteria were: 1) liver or kidney dysfunction; 2) positive for hepatitis B virus; 3) known allergy to the investigational product; 4) systemic chronic disease or immune deficient; 5) diagnosed with, or at high risk of, malignancy; 6) mental illness; 7) diagnosed with other growth and development disorders (GH deficiency, Turner syndrome, Noonan syndrome, Laron syndrome, small for gestational age, or growth disorders caused by malnutrition or hypothyroidism, or short stature of other known causes); 8) impaired glucose regulation or diabetes; 9) body mass index ≥22 kg/m^2; 10) congenital skeletal abnormalities, scoliosis, or claudication; 11) participated in other clinical trials within 3 months; 12) received medication or other hormones that may interfere with GH secretion or function; and 13) deemed inappropriate by the study investigators. Magnetic resonance imaging (MRI) scans of the pituitary gland were conducted to exclude pituitary tumors.

2.2 Study Design

This phase 3 study consisted of 2 phases. The first phase was a 52-week, multicenter, randomized, controlled, open-label study and the second phase conducted after the first year for another 52 weeks was an extended, open-label, observational study. The

Abbreviations: $\triangle$, change in; AE, adverse event; ANCOVA, analysis of covariance; BA, bone age; CA, chronological age; CI, confidence interval; CSPEM, Chinese Society of Pediatric Endocrinology and Metabolism; ECG, electrocardiogram; FAS, full analysis set; FSS, familial short stature; GH, growth hormone; GH-IGF-1, growth hormone-insulin-like growth factor 1; IGFBP-3, IGF-binding protein 3; ITT, intention-to-treat; HT-SDS, height standard deviation score; HV, height velocity; ISS, idiopathic short stature; LSM, least squares mean; MRI, magnetic resonance imaging; NFSS, nonfamilial short stature; PAH, predicted adult height; PPS per-protocol set; rhGH, recombinant human GH; SAE, serious adverse event; SD, standard deviation; SDS, standard deviation score; SHOX, short stature homeobox-containing; SS, safety set; TEAE, treatment-emergent adverse event.

study was conducted at 11 clinical sites in China. Here, we report the first phase study results from baseline up to week 52.

In the first phase, all subjects were randomized 3:1 to daily subcutaneous injections of rhGH 0.05 mg/kg/day (Jintropin®, GeneScience Pharmaceuticals, Changchun, China) or no treatment for 52 weeks or until unacceptable toxicity or investigator decision. There was no positive control group in this study because GH was not approved for ISS in China at the start of this study. Block randomization method was performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). All subjects eligible for the study were given a random number in the order of enrollment, and a central randomization system was used to determine whether the subject was allocated to the treatment or control group. The random unique identifier generated for each subject was used throughout the study.

According to the U.S. FDA, the maximum dose of GH approved for the treatment of ISS in children is 0.47 mg/kg/week (equivalent to 0.067 mg/kg/day or 0.2 IU/kg/day). The Chinese Society of Pediatric Endocrinology and Metabolism (CSPEM) recommends children with ISS should receive rhGH at a dose of 0.043–0.07 mg/kg/day, equivalent to 0.125–0.2 IU/kg/day (9). In this study, children in the treatment group were given somatropin 0.05 mg/kg/day subcutaneously, which was equivalent to 0.15 IU/kg/day, lower than the U.S. approved dose and within the CSPEM recommended dose.

The study was carried out according to the Declaration of Helsinki and complied with the standards of Good Clinical Practice. Written informed consent from subjects, parents, or guardians was obtained prior to enrollment. The protocol was reviewed and approved by the Ethics Committee of each investigation site.

2.3 Outcomes and Assessment

All subjects underwent a total of 6 visits to the clinic throughout the first phase of the study at baseline and weeks 4, 13, 26, 39, and 52. The primary objective of this study was to compare the treatment improvement in HT-SDS with control at week 52. The secondary objective was to determine the improvement in annual HV at week 52 with treatment.

The primary outcome measure was △HT-SDS for chronological age (CA) from baseline at week 52. Other secondary outcome measures included △HT-SDS for CA at weeks 4, 13, 26, and 39; △height from baseline; △BA/CA; △HV; and △IGF-1 SDS. Safety was monitored throughout the study and assessed based on reported AEs, physical examinations, vital signs, laboratory test results (e.g., blood, urine, antidrug antibodies, thyroid function, fasting blood glucose), whole-spine X-rays, and electrocardiograms (ECGs).

GH stimulation tests and pituitary MRIs were performed within 1 year before randomization at the investigation site where the subjects were screened. All other tests were performed within 8 weeks before randomization at the participating site. Predicted adult height (PAH) was also assessed using the China05 method (The Standards of Skeletal Maturity of Hand and Wrist for Chinese–China 05 and its application) (10). BA radiography was performed using the TW3-AI method (11) and the results were collated and analyzed by a qualified researcher appointed by the principal investigator at the Children's Hospital of Zhejiang University School of Medicine. IGF-1 and IGF-binding protein 3 (IGFBP-3) serum were analyzed at a central laboratory. Subjects in the treatment group were screened for antidrug and neutralizing antibodies at baseline and weeks 26 and 52.

2.4 Statistical Analysis

All statistical analyses in the first phase of the study were performed using SAS version 9.4.

This study was designed to demonstrate the superiority of somatropin versus no treatment in terms of improving HT-SDS. Based on previous research and the investigators' decision, the predetermined difference in mean change of HT-SDS in the experimental and control groups after 52 weeks was set at $\delta = 0.5$. Assuming a combined variance of 1.44, type I error $\alpha = 0.025$, type II error $\beta = 0.15$, and a power of 0.85, the required sample sizes for the treatment and control groups were not to be less than 210 and 70 subjects, respectively. To ensure that the results were statistically robust, conformed to the minimum number of patients for a phase 3 study required by China's National Medical Products Administration, and accounted for a 20% dropout rate, a total of 480 subjects (somatropin: 360; untreated control: 120) were recruited.

The full analysis set (FAS) of the first study phase was defined as all subjects who received at least 1 dose of study drug, had baseline assessments, and had at least 1 postbaseline assessment evaluated after randomization, according to the intention-to-treat (ITT) principle. All missing data were imputed using the last-observation-carried-forward method. The per-protocol set (PPS) was a subset of the FAS that included all subjects without any major protocol deviations. The FAS was the main data set for the evaluation of efficacy. Both ITT and PPS were analyzed to prevent selection bias. Safety data analyses were performed on a safety set (SS) that included all subjects who had received the study drug at least once in the treatment group and all subjects in the control group after randomization.

Data were presented as mean ± SD for quantitative and efficacy variables, and frequency and percentage for qualitative variables. Descriptive statistics were used to summarize baseline characteristics. $P < 0.05$ was considered statistically significant. Within-group comparisons were assessed using the paired t test and Wilcoxon rank-sum test. Intergroup comparisons were performed using analysis of covariance (ANCOVA). The change from baseline efficacy endpoints at week 52 was tested for superiority of somatropin to no treatment using least squares mean (LSM) difference. There is evidence of superiority if the 95% confidence interval (CI) for the treatment effect lies entirely above zero. AEs were summarized descriptively by severity and relationship to somatropin.

A sensitivity analysis was performed in the first phase with baseline HT-SDS and study group as the fixed effects, and the center was used as the random effect. Comparison between groups was performed using the mixed-effects model. The factors associated with ΔHT-SDS, ΔHV, and ΔPAH in response to GH treatment were determined in separate multivariate linear regression analyses.

3 RESULTS

3.1 Subject Baseline Characteristics and Demographics

A total of 592 subjects were screened, of whom 481 were randomized 3:1 to somatropin (n = 362) and untreated control (n = 119) (**Figure 1**). Three hundred and fifty-one (97.0%) and 108 (90.8%) subjects in the treatment and control groups, respectively, competed the study. Twenty-two subjects (11 from each group) dropped out early, the most common reason being "withdrawal of consent" (n = 12). The numbers of subjects included in the FAS, PPS, and SS were 472 (98.1%), 459 (95.4%), and 481 (100%), respectively.

The demographic information and baseline characteristics of the study subjects are presented in **Table 1**. The mean CA of the subjects was 5.8 ± 1.55 years (range: 4.0–9.0 years). The mean height was 106.0 ± 8.14 cm, the mean weight was 17.0 ± 2.93 kg, and the mean body mass index was 15.1 ± 1.26. Baseline HT-SDS was similar in both the treatment and control groups (somatropin: –2.64 ± 0.41; untreated control: –2.67 ± 0.44). Pretreatment HV did not differ between the groups. The percentage of subjects who were compliant with treatment was 98.03% ± 2.45%.

3.2 Primary and Secondary Endpoints

3.2.1 HT-SDS and △HT-SDS

At week 52, the HT-SDSs in the treatment and control groups were –1.60 ± 0.53 and –2.48 ± 0.54, respectively (**Figure 2A**). The mean △HT-SDS at week 52 relative to baseline was 1.04 ± 0.31 in the treatment group and 0.20 ± 0.33 in the control group, showing a statistically significant difference between the 2 groups ($P < 0.001$, **Table 2**). △HT-SDS at all evaluable time points from baseline was statistically significant for both study groups ($P < 0.001$). The LSM difference in △HT-SDS at week 52 between the treatment and control groups was 0.85 (95% CI 0.78–0.91), indicating superiority of treatment over control. Subjects in the treatment group converged toward the normal range (HT-SDS ≥ –2) at the end of 52 weeks. Greater ΔHT-SDS at week 52 was observed in children aged ≤7 years than those who were aged >7 years (1.08 ± 0.31 vs. 0.85 ± 0.24).

Efficacy in the PPS also showed similar results. HT-SDS was –1.58 ± 0.51 and –2.64 ± 0.41 in the treatment and control groups, respectively. Mean △HT-SDS was 1.06 ± 0.30 with treatment and 0.18 ± 0.27 in the control group, with the difference between both groups statistically significant ($P < 0.001$). The LSM difference in △HT-SDS between treatment and control was 0.88 (95% CI 0.82–0.94).

3.2.2 Height

Increase in mean height was observed in both study groups across all evaluable time points (**Figure 2B**), with those in the treatment group experiencing larger gain in height compared with untreated subjects. △height was statistically significantly higher in the treatment group compared with control at all time points ($P < 0.001$). At week 52, mean △height from baseline in the treatment group was 10.19 ± 1.47 cm and 5.85 ± 1.80 cm in the control group (**Table 2**). The LSM difference in the △height between the treatment and control groups was 4.27 (95% CI 3.95–4.59).

3.2.3 BA/CA

At week 52, the BA/CA ratios were 0.85 ± 0.13 and 0.82 ± 0.14 in the treatment and control groups, respectively (**Figure 2C**). The mean △BA/CA ratio significantly increased from baseline at week 52 with treatment (0.04 ± 0.09; $P < 0.001$, **Table 2**). There was a significant difference between groups in △BA/CA at week 52 ($P < 0.001$); the LSM difference between the treatment and control groups at weeks 26 and 52 was 0.014 (95% CI –0.0003 to 0.0287) and 0.035 (95% CI 0.017–0.052), respectively. At week 52, 95% CI was more than 0, indicating that treatment had an effect on bone maturation.

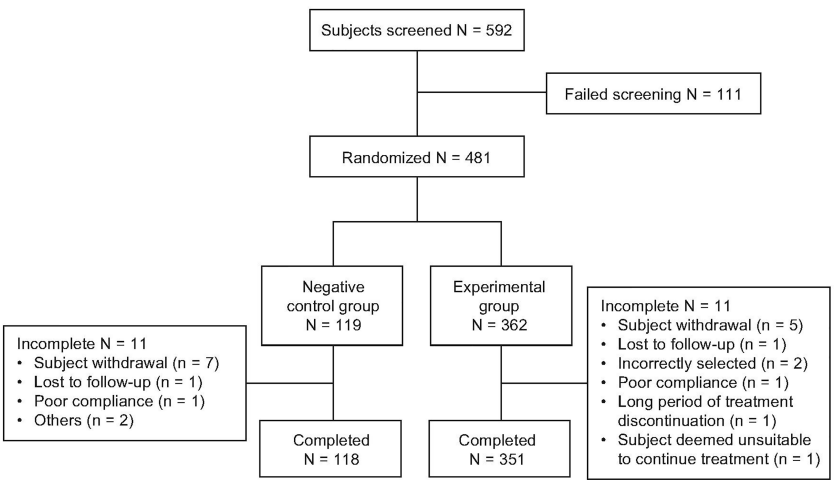


FIGURE 1 | Patient flow throughout the trial.

TABLE 1 | Patient demographics and baseline characteristics of the FAS.

	Untreated control (n = 119)	Somatropin 0.05 mg/kg/day (n = 362)	Total (N = 481)
Chronological age, year	6.0 ± 1.67	5.8 ± 1.51	5.8 ± 1.55
Gender			
Male, n (%)	63 (56.3)	224 (62.2)	287 (60.8)
Female, n (%)	49 (43.8)	136 (37.8)	185 (39.2)
Height, cm	106.81 ± 8.81	105.75 ± 7.91	106.00 ± 8.14
Weight, kg	17.30 ± 3.02	16.90 ± 2.90	17.00 ± 2.93
BMI, kg/m^2	15.10 ± 1.27	15.10 ± 1.27	15.10 ± 1.26
Ethnicity			
Han (%)	108 (96.4)	350 (97.2)	458 (97.0)
Others (%)	4 (3.6)	10 (2.8)	14 (3.0)
HT-SDS	−2.67 ± 0.44	−2.64 ± 0.41	−2.65 ± 0.42
BA/CA	0.81 ± 0.15	0.81 ± 0.15	0.81 ± 0.15
HV, cm/year	5.46 ± 5.10	5.00 ± 3.38	5.06 ± 3.85
IGF-1 SDS	−0.72 ± 0.95	−0.49 ± 0.99	–

BA, bone age; BMI, body mass index; CA, chronological age; FAS, full analysis set; HT-SDS, height standard deviation score; HV, height velocity; IGF-1 SDS, insulin-like growth factor-1 standard deviation score.

3.2.4 HV

Mean HV increased sharply in the first month of treatment from 4.97 ± 3.38 cm/year at baseline to 12.40 ± 7.05 cm/year at week 4, before plateauing (**Figure 2D**). Interestingly, a similar trend was also observed in the control group (**Figure 2D**). The annualized HVs at week 52 in the treatment and control groups were 10.18 ± 1.47 cm/year and 5.81 ± 1.68 cm/year, respectively (**Figure 2D**). △HVs at 52 weeks were 5.17 ± 3.70 cm/year and 0.75 ± 4.34 cm/

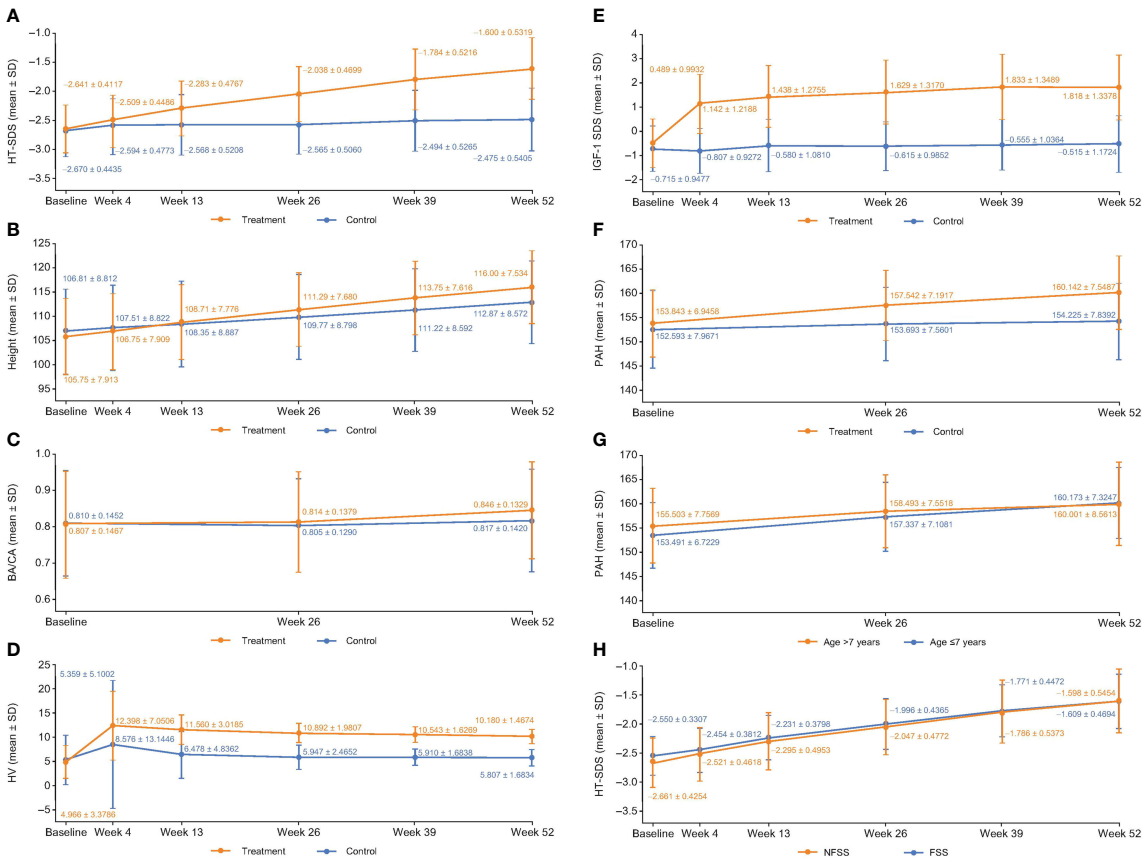


FIGURE 2 | **(A)** HT-SDS, **(B)** height, **(C)** BA/CA, **(D)** HV, **(E)** ΔIGF-1 SDS, **(F)** PAH, **(G)** PAH stratified by age and, **(H)** HT-SDS for FSS and NFSS of the FAS at each evaluable time point. Δ, change in; BA, bone age; CA, chronological age; FAS, full analysis set; FSS, familial short stature; HT-SDS, height standard deviation score; HV, height velocity; IGFBP-3, insulin-like growth factor binding protein 3; IGF-1 SDS, insulin-like growth factor-1 standard deviation score; NFSS, nonfamilial short stature; PAH, predicted adult height; SD, standard deviation.

TABLE 2 | Efficacy outcome measures of the FAS.

	Untreated control (n = 112)	Somatropin 0.05 mg/kg/day (n = 360)
ΔHT-SDS		
Week 4	0.08 ± 0.22	0.13 ± 0.14
Week 13	0.10 ± 0.28	0.36 ± 0.21
Week26	0.11 ± 0.25	0.60 ± 0.22
Week39	0.18 ± 0.30	0.86 ± 0.28
Week52	0.20 ± 0.33	1.04 ± 0.31
ΔHeight, cm		
Week4	0.70 ± 1.05	1.00 ± 0.58
Week13	1.67 ± 1.22	2.93 ± 0.80
Week26	3.04 ± 1.24	5.51 ± 1.02
Week39	4.51 ± 1.35	7.98 ± 1.25
Week52	5.85 ± 1.80	10.19 ± 1.47
ΔPAH, cm		
Week26	1.13 ± 4.09	3.66 ± 4.08
Week52	1.55 ± 4.96	6.28 ± 4.84
ΔBA/CA		
Week26	–0.007 ± 0.070	0.009 ± 0.070
Week52	0.004 ± 0.100	0.040 ± 0.090
ΔHV, cm/year		
Week4	3.12 ± 14.83	7.43 ± 8.39
Week13	1.05 ± 7.57	6.58 ± 4.92
Week26	0.55 ± 5.97	5.91 ± 4.10
Week39	0.51 ± 5.61	5.56 ± 3.81
Week52	0.75 ± 4.34	5.17 ± 3.70
ΔIGF-1 SDS		
Week4	–0.10 ± 0.71	1.62 ± 0.92
Week13	0.15 ± 1.02	1.92 ± 1.06
Week26	0.10 ± 0.79	2.12 ± 1.11
Week39	0.15 ± 0.87	2.33 ± 1.15
Week52	0.22 ± 0.98	2.31 ± 1.20

Δ, change in; BA, bone age; CA, chronological age; FAS, full analysis set; HT-SDS, height standard deviation score; HV, height velocity; IGF-1 SDS, insulin-like growth factor-1 standard deviation score; PAH, predicted adult height.

year in the treatment and control groups, respectively (**Table 2**). Compared with baseline, all study groups were associated with a significant increase in HV at all time points. The LSM of △HV at week 52 between the treatment and control groups was 4.42 cm/year (95% CI 4.10–4.75), demonstrating superiority in terms of increment in HV with treatment.

There was not much difference in HV between children aged ≤7 years and those aged >7 years (5.17 ± 3.89 cm/year vs. 5.16 ± 2.63 cm/year). However, the LSM difference between >7-year-olds and ≤7-year-olds in terms of ΔHV by ANCOVA was –1.12 cm/year (95% CI –1.48 to –0.75).

3.2.5 IGF-1 SDS

IGF-1 SDS increased sharply from –0.49 ± 0.99 at baseline to 1.14 ± 1.22 at week 4 with treatment and progressed steadily before plateauing at week 39 (**Figure 2E**). At week 52, statistically significant △IGF-1 SDS from baseline was observed in the treatment (2.31 ± 1.20; $P < 0.001$) and control groups (0.22 ± 0.98; $P = 0.021$) (**Table 2**). Treatment differed significantly compared with control at all time points ($P < 0.001$); the LSM difference between treatment and control was 2.16 (95% CI 1.92–2.41) at week 52. The 95% CI of LSM difference was more than 0 from week 4 through week 52, indicating superiority in the treatment group throughout the study.

3.3 Additional Assessment

3.3.1 PAH

PAH was analyzed since the study did not follow up with the subjects until adult height was achieved. The mean PAH at the start of treatment in the treatment group was 153.84 ± 6.95 cm and reached a mean of 160.14 ± 7.55 cm at the end of treatment ($P < 0.001$; **Figure 2F**). In contrast, the PAH for subjects in the control group was 152.59 ± 7.96 cm at baseline and 154.22 ± 7.84 cm at 52 weeks ($P = 0.002$; **Figure 2F**). ΔPAHs in the treatment and control groups were 6.28 ± 4.81 cm and 1.55 ± 4.96 cm, respectively (**Table 2**). The LSM in ΔPAHs at week 52 between the treatment and control groups was 4.93 (95% CI 3.91–5.96).

PAH was further stratified by age to assess if age of initiation had an impact on the efficacy of GH treatment. ΔPAH at week 52 was 6.67 ± 4.96 cm in children aged ≤7 years and 4.50 ± 3.81 cm in children aged >7 years, suggesting that a greater gain in height was observed in children aged ≤7 years (**Figure 2G**). The LSM difference in ΔPAH between the treatment and control groups in children aged ≤7 years was 5.51 (95% CI 4.32–6.70) and 2.77 (95% CI 0.90–4.65) in children aged >7 years.

3.3.2 Familial Short Stature

ISS is a heterogenous condition covering children with familial short stature (FSS) and nonfamilial short stature (NFSS). FSS is defined as a child with short stature compared with the relevant

population, but remains within the expected target height range for the family, with 1 parent HT-SDS < –2 (1). Here, subgroups of FSS and NFSS were analyzed to determine the impact of GH treatment. At 52 weeks, in GH-treated subjects, ΔHT-SDS from baseline was 0.94 ± 0.30 in the FSS group and 1.06 ± 0.31 in the NFSS group (both $P < 0.001$). In the FSS group, HT-SDS was –1.61 ± 0.47 and –2.40 ± 0.45 in the treatment and control groups, respectively, whereas in the NFSS group, HT-SDS was –1.60 ± 0.55 and –2.49 ± 0.56 in the treatment and control groups, respectively, at week 52. **Figure 2H** compares the HT-SDS between the FSS and NFSS groups. There was significant improvement in terms of HT-SDS ($P < 0.001$) in the NFSS group compared with the FSS group at the end of 52 weeks (**Figure 2H**). The LSM difference in ΔHT SDS between FSS and NFSS was –0.11 (95% CI –0.19 to –0.04). PAH was 4.99 ± 4.88 and 0.14 ± 4.03 in the FSS treatment and control groups, respectively, whereas in the NFSS group, PAH was 6.56 ± 4.80 and 1.83 ± 5.10 in the treatment and control groups, respectively, at week 52. The LSM difference in ΔPAH at week 52 between FSS and NFSS was –1.36 (95% CI –2.49 to –0.23). Similar trends were also observed with HV at 52 weeks, with the FSS group and the NFSS group achieving mean HV of 9.75 ± 1.46 cm/year and 10.27 ± 1.45 cm/year with treatment, respectively. The LSM difference between FSS and NFSS in ΔHV at week 52 was –0.48 (95% CI –0.86 to –0.10).

3.4 Safety

The frequencies of total treatment-emergent adverse events (TEAEs) were similar in both study groups (treatment vs. control: 89.8% vs. 82.4%, **Table 3**). Most TEAEs were mild to moderate in severity. The most common TEAEs in the treatment group were upper respiratory tract infection (66.0%), fever (19.6%), cough (10.8%), bronchitis (5.5%), respiratory tract infection (7.2%), rhinitis (2.8%), and indigestion (2.5%). One subject withdrew from the study due to neutropenia, which was deemed unrelated to treatment.

AEs reported in 23 (6.4%) subjects in the treatment group were considered related to treatment. They were all mild to moderate in severity. Of note, 4 subjects experienced elevated thyroid-stimulating hormone, 3 had scoliosis, elevated blood glucose level and rash occurred in 2 subjects each, and hypersensitivity and hypothyroidism were reported in 1 subject each. All but 3 subjects (1 case of hypothyroidism, scoliosis, and extremity pain each) recovered from the drug-related TEAEs. Serious AEs (SAEs) occurred in 25 subjects (treatment: 19 [5.2%], control: 6 [5.0%]) and were deemed unrelated to treatment. All subjects recovered from the SAEs. No deaths were reported with GH treatment in the study.

The numbers of subjects with IGF-1 SDS more than +2 in the treatment and control groups at week 52 were 154 (42.5%) and 2 (1.7%), respectively. IGF-1/IGFBP-3 ratios in the treatment and control groups were 0.19 ± 0.05 and 0.13 ± 0.04, respectively. Abnormal ECGs were reported in 7 (1.9%) subjects in the treatment group and 2 (1.6%) subjects in the control group. Whole-spine X-ray examination was deemed abnormal in 13 subjects in the treatment group and 4 subjects in the control group. There were generally no unexpected safety issues with respect to clinical laboratory examinations, vital signs, and physical examinations.

The numbers of subjects detected with antidrug antibodies at baseline, week 26, and 52 were 0 (0.0%), 5 (1.4%), and 10 (2.8%), respectively; they all tested negative for neutralizing antibodies.

4 DISCUSSION

Several studies have been conducted to investigate the benefits of rhGH therapy in children with ISS; however, only a few were randomized with a negative control or placebo to compare the effect of treatment on height outcomes (12–15). The purpose of this study was to evaluate the safety and efficacy of daily somatropin at a dose of 0.05 mg/kg/day in Chinese children with ISS, and to assess the superiority of treatment over control. Overall, the results for the PPS were consistent with the FAS across all outcome measures, confirming the robustness of these data. The parallel study design and large number of children recruited made this study one of the few that provided objective

TABLE 3 | Adverse events of the SS.

	Untreated control (n = 119) n (%)	Somatropin 0.05 mg/kg/day (n = 362) n (%)	Total (N = 481) n (%)
Total TEAEs	98 (82.4)	325 (89.8)	426 (88.6)
Total TRAEs	0 (0.0)	23 (6.4)	23 (4.8)
SAEs	6 (5.0)	19 (5.2)	25 (5.2)
Treatment suspension due to TEAEs	0 (0.0)	203 (56.1)	203 (42.2)
Treatment suspension due to TRAEs	0 (0.0)	2 (0.6)	2 (0.4)
Treatment suspension due to SAEs	0 (0.0)	13 (3.6)	13 (2.7)
TEAEs occurring in ≥5% of subjects in any group			
Upper respiratory tract infection	78 (65.5)	239 (66.0)	317 (65.9)
Fever	10 (8.4)	71 (19.6)	81 (16.8)
Cough	10 (8.4)	39 (10.8)	49 (10.2)
Bronchitis	12 (10.1)	20 (5.5)	32 (6.7)
Respiratory tract infection	5 (4.2)	26 (7.2)	31 (6.4)
Rhinitis	8 (6.7)	10 (2.8)	18 (3.7)
Indigestion	7 (5.9)	9 (2.5)	16 (3.3)

SAEs, serious adverse events; SS, safety set; TEAEs, treatment-emergent adverse events; TRAEs, treatment-related adverse events.

evidence of the effects of daily rhGH treatment in the Asian population.

Greater improvement in HT-SDS was observed in the treatment group than in the control group at week 52 (−1.60 ± 0.53 vs. −2.48 ± 0.54). The mean HT-SDS of subjects who received somatropin reached normal range (≥ −2.25) after a year of treatment, indicating that GH treatment has a positive impact on growth. These subjects achieved a mean height of 3.13 cm taller than untreated subjects. In a meta-analysis of 6 randomized and 4 nonrandomized controlled trials evaluating the effect of short-term GH therapy in children with ISS, the difference in HT-SDS between the treatment and control group was reported to be 0.60 after a year (16). HV was significantly greater in the GH-treated group than in controls after 1 year of treatment, with the pooled estimate for the difference between both groups being 2.86 ± 0.37 cm/year (16). Of note, the age ranges of the analyzed children were older, with a few studies including pubertal children. This meta-analysis provided evidence that 1 year of GH therapy can increase HV and HT-SDS.

The results of our study were consistent with more recent studies in the Asian population, showing improvements in auxological variables in terms of HT-SDS and HV. In a phase 3, randomized, controlled trial, Chung et al. reported an increase in 6-month HV from 5.63 ± 1.62 cm/year at baseline to 10.08 ± 1.92 cm/year with Saizen® 0.067 mg/kg/day. The difference in △HV between treatment and control was 3.47 cm/year (95% CI 2.51–5.00; $P < 0.0001$). △HT-SDS was 0.96 at 12 months with treatment (12). Although the subjects recruited were slightly older and the treatment dose was higher, their results were comparable with ours. Similar benefits were observed in another phase 3 study conducted by Kim et al. (14). In this study, HV and HT-SDS were 10.68 ± 1.95 cm/year and 0.63 ± 0.16, respectively, after 6 months of Growtropin®-II 0.37 mg/kg/week, which was equivalent to 0.05 mg/kg/day (14). In an open-label study, Eutropin® 0.37 mg/kg/week for 26 weeks was able to achieve a HV of 6.36 ± 3.36 cm/year and the gain in HT-SDS was 0.57 ± 0.27 (17). The short-term benefit of GH treatment was also demonstrated in a real-world observational study of 2,596 subjects with ISS with a mean age of 11.5 years and a baseline HT-SDS of −2.3 ± 0.8 (18). 1-year of GH treatment improved HT-SDS by 0.61 ± 0.33 (18). These studies demonstrated that short-term GH treatment was able to achieve growth enhancement in prepubertal children with ISS.

Daily administration of rhGH in prepubertal children with ISS during the 52-week treatment period significantly increased ΔPAH, HV, and IGF-1 SDS compared with control at all time points. Of note, the improved PAH could be taken to denote that short-term treatment had a beneficial effect on final height. While BA/CA increased with GH treatment compared with control, it remained <1 after 52 weeks of treatment. Given the large sample size, it was easier to achieve statistical significance with bone maturation as it increased from 0.81 ± 0.15 at baseline to 0.85 ± 0.13 at week 52. △BA/CA was 0.04 ± 0.09, indicating slight progression of BA. The LSM difference also showed that GH treatment increased △BA/CA compared with control. However, 1 study from Korea reported no significant difference in ΔBA from baseline with treatment compared with control (14). The effect of treatment on BA/CA varies, 1 study reported a ratio of 1.06 ± 1.00 after 3 years of treatment, while another 0.93 ± 0.11 with 1 year of treatment (19, 20). The inclusion of children during peripuberty or puberty may influence BA progression and BA/CA ratio because of the effects of sex steroids. In our study, all enrolled children were prepubertal at baseline, and only 19 (4.0%; 13 [3.6%] in the treatment group and 6 [5.4%] in the control group) advanced to a higher Tanner stage by week 52, which was unlikely to confound the efficacy assessments. Nonetheless, a delay in BA at the onset of treatment was associated with greater BA progression in the first year of therapy and longer-term treatment may gradually increase BA/CA ratio to 1, enabling BA to catch up with CA (21, 22).

Subjects were further stratified by age. Children aged 7 years or younger showed larger increments in ΔHT-SDS, △HV, and ΔPAH compared with those who were older than 7 years, suggesting that starting GH treatment earlier may yield better growth outcomes. Further study is warranted to elucidate the optimal age of treatment. In a retrospective cohort study by Ranke et al., height achieved and gain in HT-SDS depended on the age at which GH was initiated, supporting the notion that starting GH treatment earlier yielded a better response (23).

It has been suggested that ISS can be subdivided into FSS and NFSS. FSS refers to children with a normal growth velocity and growing in a normal trajectory toward their midparental height range but are short compared to the reference population (24). In our subgroup analysis, treated subjects categorized as NFSS had a higher HT-SDS, PAH, and HV compared with those in the FSS group. Similarly, Sotos and Tokar conducted a retrospective analysis to compare FSS and NFSS, and reported a favorable height gain in the latter (25). Similarly, earlier studies by Wit et al. and Albertsson-Wikland et al. also showed that children with NFSS responded better to GH treatment than those with FSS (26, 27), as FSS is a condition believed to be caused by small contributions of multiple genes. The smaller benefit observed in children with FSS compared with NFSS may be attributed to lower GH sensitivity, GH resistance, or mutations in the IGF-1 gene (24). Based on this sub-analysis, it may be useful to categorize subjects with ISS into FSS and NFSS so as to better predict their growth outcomes with GH treatment. Further research is warranted to validate this observation.

Daily somatropin 0.05 mg/kg/day had a favorable safety profile throughout the 52-week study period, apart from the subject who experienced elevated alanine aminotransferase. The subject was treated with cough syrup prior to the GH treatment and there were no other abnormal signs or symptoms. It was not clear whether the increase in alanine aminotransferase was due to an infection or the cough syrup, nevertheless, the subject recovered without needing further intervention. In 1 study, Quigley et al. also observed AEs such as scoliosis, hypothyroidism, and changes in carbohydrate metabolism with GH treatment in pediatric patients with ISS based on a dosing range of 0.22–0.37 mg/kg/week (28). Interestingly, otitis media, which was not present in our study, was reported in 8% of patients (28). Leschek et al. reported scoliosis in 7 patients who

received GH treatment at a dose of 0.22 mg/kg/wk, 3 more than placebo (15). Another study reported mild pruritus on the injection site, which was absent in this present study, as the only adverse event related to treatment spontaneously resolved without any intervention (17). The incidences of TEAEs reported in both study groups were similar. All SAEs that occurred during the study, such as upper respiratory tract infection, bronchitis, and tonsilitis, were deemed unrelated to the treatment. Elevated IGF-1 levels have been associated with the development of cancer (29). While the mean IGF-1 SDS in the treatment group of our study was 1.82 ± 1.34 at week 52, 42.5% of subjects had IGF-SDS levels of more than +2, which was above the normal range using age-appropriate reference standards. Although there has been no evidence so far to suggest increased risk of some cancers in later life with the use of GH, it is appropriate to monitor IGF-1 levels and adjust and tailor the dose where necessary, especially for those who are receiving long-term treatment. Antidrug antibodies to somatropin that were detected in some children had no effect on the efficacy and safety of treatment, as their data showed consistent results with those of antibody-negative subjects.

The strength of this study is the inclusion of a negative control group, giving confidence that any efficacy and safety effects may be attributable to daily somatropin at 0.05 mg/kg/day. While randomized, controlled trials provide the most robust evidence, they may not reflect what occurs in the real world. Hence, the ongoing, open-extension, observational study will shed light on whether the clinical benefit of somatropin is sustained following another year of individualized treatment.

There are several limitations of this study. It does not present a full picture of short-term treatment on adult height. It also does not elucidate the long-term efficacy of somatropin. As such, longer treatment and follow-up are warranted. This is supported by a recent observational study in China where a longer GH-treatment course of ≥2 years yielded better efficacy compared with shorter treatment courses in terms of △HT-SDS in children with ISS, despite administering during peripuberty (≥2 years vs. 1–2 years vs. 6–12 months vs. 3–6 months: 1.54 ± 1.23 vs. 1.01 ± 1.31 vs. 1.00 ± 1.27 vs. 1.30 ± 1.09) (6). The extended, open-label, observation study of this phase 3 trial will provide a better understanding on the longer-term effects of somatropin in children with ISS in China.

In conclusion, daily somatropin at a dose of 0.05 mg/kg/day demonstrated superiority to no treatment in terms of gain in HT-SDS and HV increment. There was a significant increase in height gain, PAH, and IGF-SDS after 52 weeks of treatment in prepubertal children with ISS. somatropin was well tolerated with a favorable safety profile.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Children's Hospital of Zhejiang University School of Medicine (No.2018-IEC-003). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JF had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. JF and JY conceptualized and designed the clinical study, compiled and analyzed the data. HW, GZ, YX, HD, WG, YL, LC, FL, YZ and HG carried out the clinical assessments. JY wrote the manuscript with critical input from the other authors. All authors approved the final version of the manuscript.

FUNDING

This research was partly funded by GeneScience Pharmaceuticals Co., Ltd. The funder did not participate in the writing of the manuscript or the collection, analysis, and interpretation of the data.

ACKNOWLEDGMENTS

The authors would like to thank the participants who took part in the study. Medical writing assistance was provided by Lawrence Law, MPH, from Parexel and was funded by GeneScience Pharmaceuticals.

REFERENCES

1. Wit JM, Clayton PE, Rogol AD, Savage MO, Saenger PH, Cohen P. Idiopathic Short Stature: Definition, Epidemiology, and Diagnostic Evaluation. *Growth Horm IGF Res* (2008) 18(2):89–110. doi: 10.1016/j.ghir.2007.11.004
2. Chen WW, Liu HX, Liu J, Yang LL, Liu M, Ma HJ. Etiology and Genetic Diagnosis of Short Stature in Children. *Zhongguo Dang Dai Er Ke Za Zhi* (2019) 21(4):381–6. doi: 10.7499/j.issn.1008-8830.2019.04.015
3. Wu S, Liu QQ, Gu W, Ni SN, Shi X, Zhu ZY. A Retrospective Analysis of Patients With Short Stature in the South of China Between 2007 and 2015. *BioMed Res Int* (2018) 2018:5732694. doi: 10.1155/2018/5732694
4. Gubitosi-Klug RA, Cuttler L. Idiopathic Short Stature. *Endocrinol Metab Clin North Am* (2005) 34(3):565–80. doi: 10.1016/j.ecl.2005.04.003
5. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, et al. Consensus Statement on the Diagnosis and Treatment of Children With Idiopathic Short Stature: A Summary of the Growth Hormone Research

Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab* (2008) 93(11):4210–7. doi: 10.1210/jc.2008-0509
6. Wu D, Chen RM, Chen SK, Liu GL, Chen LQ, Yang Y, et al. Final Adult Height of Children With Idiopathic Short Stature: A Multicenter Study on Gh Therapy Alone Started During Peri-Puberty. *BMC Pediatr* (2020) 20(1):138. doi: 10.1186/s12887-020-02034-8
7. Ying YQ, Hou L, Liang Y, Wu W, Luo XP. Efficacy and Safety of Recombinant Human Growth Hormone in Treating Chinese Children With Idiopathic Short Stature. *Growth Horm IGF Res* (2018) 42-43:80–5. doi: 10.1016/j.ghir.2018.09.003
8. Zong XN, Li H. Construction of a New Growth References for China Based on Urban Chinese Children: Comparison With the Who Growth Standards. *PloS One* (2013) 8(3):e59569. doi: 10.1371/journal.pone.0059569
9. Yang F. Recommendations on the Clinical Application of Recombinant Human Growth Hormone in Pediatrics. *Chin J Women Children's Clin Med* (2014) 10(2):141–4. doi: 10.3877/cma.j.issn.1673-5250.2014.02.004
10. Zhang S, Ma Z, Shen X. The Standards of Skeletal Maturity of Hand and Wrist for Chinese-China 05 IV. The Characteristics of Skeletal Development in Chinese Children.. *Chin J Sports Med* (2007) 26(4):452–5. doi: 10.16038/j.1000-6710.2007.04.014
11. Zhou XL, Wang EG, Lin Q, Dong GP, Wu W, Huang K, et al. Diagnostic Performance of Convolutional Neural Network-Based Tanner-Whitehouse 3 Bone Age Assessment System. *Quant Imaging Med Surg* (2020) 10(3):657–67. doi: 10.21037/qims.2020.02.20
12. Chung WY, Yoo HW, Hwang JS, Ko CW, Kim HS, Jin DK, et al. Effect of Growth Hormone Therapy on Height Velocity in Korean Children With Idiopathic Short Stature: A Phase Iii Randomised Controlled Trial. *Horm Res Paediatr* (2018) 90(1):44–53. doi: 10.1159/000491016
13. Genentech Collaborative Study Group. Idiopathic Short Stature: Results of a One-Year Controlled Study of Human Growth Hormone Treatment. *J Pediatr* (1989) 115(5 Pt 1):713–9. doi: 10.1016/s0022-3476(89)80647-x
14. Kim J, Suh BK, Ko CW, Lee KH, Shin CH, Hwang JS, et al. Recombinant Growth Hormone Therapy for Prepubertal Children With Idiopathic Short Stature in Korea: A Phase Iii Randomized Trial. *J Endocrinol Invest* (2018) 41(4):475–83. doi: 10.1007/s40618-017-0786-8
15. Leschek EW, Rose SR, Yanovski JA, Troendle JF, Quigley CA, Chipman JJ, et al. Effect of Growth Hormone Treatment on Adult Height in Peripubertal Children With Idiopathic Short Stature: A Randomized, Double-Blind, Placebo-Controlled Trial. *J Clin Endocrinol Metab* (2004) 89(7):3140–8. doi: 10.1210/jc.2003-031457
16. Finkelstein BS, Imperiale TF, Speroff T, Marrero U, Radcliffe DJ, Cuttler L. Effect of Growth Hormone Therapy on Height in Children With Idiopathic Short Stature: A Meta-Analysis. *Arch Pediatr Adolesc Med* (2002) 156(3):230–40. doi: 10.1001/archpedi.156.3.230
17. Kim HS, Yang SW, Yoo HW, Suh BK, Ko CW, Chung WY, et al. Efficacy of Short-Term Growth Hormone Treatment in Prepubertal Children With Idiopathic Short Stature. *Yonsei Med J* (2014) 55(1):53–60. doi: 10.3349/ymj.2014.55.1.53
18. Child CJ, Quigley CA, Cutler GB Jr, Moore WV, Wintergerst KA, Ross JL, et al. Height Gain and Safety Outcomes in Growth Hormone-Treated Children With Idiopathic Short Stature: Experience From a Prospective Observational Study. *Horm Res Paediatr* (2019) 91(4):241–51. doi: 10.1159/000500087
19. Im M, Kim YD, Han HS. Effect of Growth Hormone Treatment on Children With Idiopathic Short Stature and Idiopathic Growth Hormone Deficiency. *Ann Pediatr Endocrinol Metab* (2017) 22(2):119–24. doi: 10.6065/apem.2017.22.2.119
20. Kang MJ, Kim EY, Shim YS, Jeong HR, Lee HJ, Yang S, et al. Factors Affecting Bone Age Maturation During 3 Years of Growth Hormone Treatment in Patients With Idiopathic Growth Hormone Deficiency and Idiopathic Short Stature: Analysis of Data From the Lg Growth Study. *Med (Baltimore)* (2019) 98(14):e14962. doi: 10.1097/MD.0000000000014962
21. Darendeliler F, Ranke MB, Bakker B, Lindberg A, Cowell CT, Albertsson-Wikland K, et al. Bone Age Progression During the First Year of Growth Hormone Therapy in Pre-Pubertal Children With Idiopathic Growth Hormone Deficiency, Turner Syndrome or Idiopathic Short Stature, and in Short Children Born Small for Gestational Age: Analysis of Data From Kigs (Pfizer International Growth Database). *Horm Res* (2005) 63(1):40–7. doi: 10.1159/000082872
22. Ross JL, Lee PA, Gut R, Germak J. Attaining Genetic Height Potential: Analysis of Height Outcomes From the Answer Program in Children Treated With Growth Hormone Over 5 Years. *Growth Horm IGF Res* (2015) 25(6):286–93. doi: 10.1016/j.ghir.2015.08.006
23. Ranke MB, Lindberg A, Price DA, Darendeliler F, Albertsson-Wikland K, Wilton P, et al. Age at Growth Hormone Therapy Start and First-Year Responsiveness to Growth Hormone are Major Determinants of Height Outcome in Idiopathic Short Stature. *Horm Res* (2007) 68(2):53–62. doi: 10.1159/000098707
24. Rajkumar V, Waseem M. *Familial Short Stature*. Treasure Island (FL: Statpearls (2021).
25. Sotos JF, Tokar NJ. Growth Hormone Significantly Increases the Adult Height of Children With Idiopathic Short Stature: Comparison of Subgroups and Benefit. *Int J Pediatr Endocrinol* (2014) 2014(1):15. doi: 10.1186/1687-9856-2014-15
26. Albertsson-Wikland K, Aronson AS, Gustafsson J, Hagenas L, Ivarsson SA, Jonsson B, et al. Dose-Dependent Effect of Growth Hormone on Final Height in Children With Short Stature Without Growth Hormone Deficiency. *J Clin Endocrinol Metab* (2008) 93(11):4342–50. doi: 10.1210/jc.2008-0707
27. Wit JM, Rekers-Mombarg LT. Dutch Growth Hormone Advisory Group. Final Height Gain by Gh Therapy in Children With Idiopathic Short Stature is Dose Dependent. *J Clin Endocrinol Metab* (2002) 87(2):604–11. doi: 10.1210/jcem.87.2.8225
28. Quigley CA, Gill AM, Crowe BJ, Robling K, Chipman JJ, Rose SR, et al. Safety of Growth Hormone Treatment in Pediatric Patients With Idiopathic Short Stature. *J Clin Endocrinol Metab* (2005) 90(9):5188–96. doi: 10.1210/jc.2004-2543
29. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-Like Growth Factor (Igf)-I, Igf Binding Protein-3, and Cancer Risk: Systematic Review and Meta-Regression Analysis. *Lancet* (2004) 363(9418):1346–53. doi: 10.1016/S0140-6736(04)16044-3

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Endocrinology

MINI REVIEW
published: 09 June 2022
doi: 10.3389/fendo.2022.902364

Growth Hormone Stimulation Testing: To Test or Not to Test? That Is One of the Questions

Mabel Yau * *and Robert Rapaport*

Division of Pediatric Endocrine and Diabetes, Mount Sinai Kravis Children's Hospital, New York, NY, United States

The evaluation of children with short stature includes monitoring over a prolonged period to establish a growth pattern as well as the exclusion of chronic medical conditions that affect growth. After a period of monitoring, evaluation, and screening, growth hormone stimulation testing is considered when the diagnosis of growth hormone deficiency (GHD) is entertained. Though flawed, growth hormone stimulation tests remain part of the comprehensive evaluation of growth and are essential for the diagnosis of growth hormone (GH) deficiency. Variables including testing length, growth hormone assay and diagnostic cut off affect results. Beyond the intrinsic issues of testing, results of GH stimulation testing can be influenced by patient characteristics. Various factors including age, gender, puberty, nutritional status and body weight modulate the secretion of GH.

Keywords: sex hormone priming, IGF – I, short stature, growth hormone deficiency, growth hormone – secretion

OPEN ACCESS

Edited by:
Valentino Cherubini,
Azienda Ospedaliero Universitaria Ospedali Riuniti, Italy

Reviewed by:
Sandro Loche,
Ospedale Microcitemico, Italy

***Correspondence:**
Mabel Yau
mabel.yau@mssm.edu

Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *23 March 2022*
Accepted: *04 May 2022*
Published: *09 June 2022*

Citation:
Yau M and Rapaport R (2022) Growth Hormone Stimulation Testing: To Test or Not to Test? That Is One of the Questions. Front. Endocrinol. 13:902364. doi: 10.3389/fendo.2022.902364

INTRODUCTION

Concern about poor growth is the leading reason for referrals to a pediatric endocrinologist (1). The evaluation of children with short stature includes monitoring over a prolonged period to establish a growth pattern as well as the exclusion of chronic medical conditions that affect growth. After a period of monitoring, evaluation, and screening, growth hormone stimulation testing is considered when the diagnosis of growth hormone deficiency (GHD) is entertained.

Lack of puberty at an expected age is another common reason patients are referred. Typically, 95% of girls present at least one sign of puberty by 13 years of age and boys by 14 years of age. These conditions of growth and pubertal delay can be difficult to diagnose because the rate of growth appears to decelerate as they cross growth percentiles around the time of the anticipated pubertal growth spurt. Thus, the growth deceleration due to constitutional delay of puberty and growth hormone deficiency can be difficult to ascertain with certainty (1).

Measuring GH concentrations at random times is unreliable because growth hormone (GH) is secreted from the anterior pituitary in a pulsatile fashion and is mainly stimulated by the release of hypothalamic growth hormone-releasing hormone (GHRH). Instead, patients are usually screened for GH deficiency by measuring serum IGF-1 and IGF-BP3 levels which have longer half-life and no pulsatility. Though flawed, growth hormone stimulation tests remain part of the comprehensive evaluation of growth and are essential for the diagnosis of growth hormone (GH) deficiency (2–4). For GH stimulation testing, two agents provoke GH secretion from the pituitary (L-dopa, clonidine,

arginine, glucagon). These provocative agents are not physiological and do not replicate normal secretory dynamics. The insulin tolerance test which is considered the gold standard for diagnosis of GHD is used to assess GH secretion in response to hypoglycemia. Given the risks associated with hypoglycemia, it is performed less frequently in the outpatient setting. Serial blood samples are taken to detect the point of maximal serum concentration of GH (1). Peak growth hormone (PGH) response to provocative testing is a vital determinant of the clinical response to GH therapy. In a study by Cohen et al. of prepubertal children characterized as GH deficient or idiopathic short stature based on GH stimulation testing without sex hormone priming, those with idiopathic short stature required doses nearly 2 times higher to reach an IGF-1 target of 2 SDS (5). The stimulated PGH level response to two pharmacologic stimuli that distinguishes between GH deficient and sufficient patients is unclear and likely exists on a continuum with levels of 5, 7 and 10 ng/mL having been proposed, each without adequate data for substantiation. The currently agreed upon peak GH cutoff is 10 ng/mL (1, 6, 7). Stimulation testing with arginine and levodopa with samples obtained for 3 hours is perhaps most frequently used in many large multicenter studies (3, 6, 8, 9).

INTRINSIC TEST FACTORS

Test Length

Some studies of various GH ST protocols suggest that sampling or duration can be reduced from 3 hours, whilst preserving the accuracy of diagnosis (10–13). Data from our single center experience of provocative GH testing using an identical protocol on a large cohort of 315 pediatric patients with short stature and/or growth failure showed peak GH response was reached by 2 hours in 97.8% of those tested. This study indicated that the GH ST with arginine and levodopa can be terminated at 2 hours without compromising its diagnostic value based on the currently accepted peak growth hormone response cutoff of 10 ng/mL, as exclusion of the 3 hour sample did not alter the GH sufficiency status in any of the 315 patients (9).

Growth Hormone Assay

There are several practical and logistical limitations to stimulation testing. Results are often often vary depending on which assay is used to analyze blood samples. Historically, GH was measured by a wide variety of approaches including bioassays, radio receptor assays, immunoassays and mass spectrometry (14). Currently, immunoassays are used most commonly to measure serum GH concentrations in clinical settings. Endogenous GH in serum exists in numerous isoforms with the majority being the isoform of 22 kDa molecular weight. However, approximately 10% circulates as the 20 kDa isoform and other isoforms and growth hormone fragments circulate in smaller portions (15). Different immunoassays can detect different spectrums of total GH isoforms. In an effort to standardize across isoforms, current consensus guidelines recommend assay calibration with a highly purified preparation of the 22 kDa recombinant human GH isoform of GH (2, 16). Additionally, GH immunoassays transitioned from using polyclonal antibodies that targeted multiple epitopes on varying GH isoforms to monoclonal antibodies targeting one isoform (14). With these changes, current assays have a narrower target.

On the new assays, GH concentrations yields are lower than on older assays. Cutoffs for peak growth hormone response to GH stimulation testing may need to be revisited with the adoption of newer assays with lower reported GH concentrations. Since only small changes in isoform ratios have been reported in certain states such has pituitary tumors and exercise, concentrations of 22 kDa GH accurately reflect total GH secretion (14). Still, inter-assay differences between immunoassays occur due to differences variations in the type of immunoassay, antibody specificity, and interference from GH binding proteins (14).

Peak Growth Hormone Cut Off

As recombinant growth hormone became more widely available, less stringent criteria for the diagnosis of GHD were implemented with increase in peak GH cut off levels. With the renewed interest in oral GH secretagogues, a reassessment of peak GH cut offs may be helpful. The studies by Bright et al. and Blum et al. suggest that a partially intact pituitary axis is needed for GH secretagogues to be effective (17, 18). Individuals with "moderate" growth hormone deficiency who may respond to GH secretagogues need to be differentiated from those with "severe" growth hormone deficiency who require growth hormone therapy (9).

Traditionally, the interpretation of GH stimulation testing results was binary with the adherence to pass/fail diagnostic GH cutoffs. Perhaps, instead, the results should be interpreted on a continuum that spans severe GHD requiring GH therapy to moderate or provisional GHD for which alternative therapies and further monitoring of growth should be considered (19). There is increasing evidence supporting the need to revisit cutoffs for peak GH after stimulation based on the assay used to measure serum GH concentrations (20, 21). Lower cutoffs for peak GH levels based on specific assays have been proposed. The establishment of method-specific clinical evidence-based GH cutoff limits would help ensure adequate clinical diagnoses.

Supporting MRI Findings

In GHD, brain MRI may show congenital pituitary abnormalities such as anterior pituitary dysplasia/hypoplasia, pituitary stalk interruption syndrome, and developmental cyst but also tumoral lesions (22). Neuroimaging is a crucial study in the diagnostic process of GHD. With only partial integrity of the hypothalamic pituitary connections, growth hormone secretion was able to be stimulated by growth hormone releasing hormone plus Arginine (23). In children with congenital GHD but less severe impairment of the pituitary stalk, the GH response to stimulation may be sufficient but pituitary GH reserve deteriorates with a GH response of < 10 ug/L after 20 yr of age (23). MRI may be helpful in differentiating those with moderate

or provisional GHD. Findings of pituitary abnormalities support decisions on GH treatment in such cases of moderate GHD (peak GH of 7-10 ng/ml), as GHD is expected to evolve.

PATIENT FACTORS

Beyond the intrinsic issues of testing, results of GH stimulation testing can be influenced by patient characteristics. GH secretion is influenced by several factors including age, gender, puberty, nutritional status and body weight (24–26).

BMI

Obesity has been associated with decreased spontaneous and stimulated GH secretion in both adults (27–30), and children (31) and weight reduction has been followed by increased GH secretion (31–35). Though the exact neuroendocrine mechanism causing the blunted GH response in obesity is unknown, proposed mechanisms include high circulating levels of insulin which can suppress GH synthesis and release and adipocyte-secreted leptin affecting GH regulation (36–39). It has been demonstrated that PGH response to stimulation testing with A-LD decreased with higher BMI SDS in a large cohort of normal weight healthy children with a range of BMI that approximated a normal distribution (mean BMI SDS of -0.3 ± 1.0). This finding suggests that the inverse relationship between BMI and PGH is not isolated to obesity and is evident in the normal weight children (9). Still, BMI is not currently consistently considered in the interpretation of the peak GH response in children.

Puberty and Sex Hormones

During puberty, there is a normal increase in growth hormone concentrations due to a larger mass of GH released per pituitary secretory episode resulting from a higher maximal rate of GH secretion per secretory burst (40). Due to the physiologic rise of GH during puberty, there is a debate as to whether prepubertal children should be "primed" with sex hormones before GH provocative testing (1). In addition to endogenous sex hormones, short term administration of exogenous sex hormones can modulate growth hormone secretion (41). Priming leads to increased peak GH levels and decreases the false positive rate for diagnosing GH deficiency in healthy controls (42–44). In the study by Marin et. al, a subset of 11 prepubertal normal children were primed with 2 days of estrogen. Peak GH response rose to levels seen in subjects at pubertal stages 4 and 5 (45). In a later study by Muller et al. of 26 boys primed with a single dose of testosterone, 77% increased their peak GH level to > 10 ng/ml (46). In a study of 315 patients undergoing GH stimulation testing, there was no difference in rates of GHD in prepubertal and pubertal patients (9).

In 2016, the Pediatric Endocrine Society updated their guidelines to support priming with sex hormones in prepubertal children (boys > 11 years old and girls > 10 years old) (2). The stated reason for this recommendation of priming was to avoid unnecessary GH treatment of children with constitutional delay of growth and puberty (2). Yet, the practice of priming remains controversial in Europe (47). In a study of 8 European countries and the US performed after these guidelines were published, priming was recommended in 5 out of 9 countries (48).

This hormonal milieu of puberty is not sustained after priming. On these supraphysiologic testosterone levels, endogenous growth hormone secretion may be overestimated. Will these children who responded to exogenous sex hormones be able to secrete enough GH at the time of puberty? Would peripubertal children who have lower peak GH levels without priming benefit from exogenous GH therapy? This overestimation can lead to false negative results and deny eligible children required treatment with growth hormone. It is unclear whether children diagnosed with GHD with or without priming respond differently to GH treatment. However, constitutional delay and GHD can be difficult to differentiate and priming should be considered in delayed puberty (49). Short term adverse side effects of priapism and testicular pain were reported in approximately 3% of prepubertal boys primed with short courses of testosterone (50).

Altering a patient's baseline characteristics is not recommended with any other stimulation testing to diagnose a hormonal deficiency. As an alternative to priming, normative values of peak growth hormone response should be further explored to develop cut off limits based on pubertal stage. This was first proposed by Rose et al. when they found that mean spontaneous night time growth hormone levels rose during pubertal development in both boys and girls, with the highest levels at mid-puberty (51). Currently, distinct cut offs are only defined for children and adults. Cut offs based on pubertal staging would bridge the continuum. Reassessment of the GH/IGF-I axis when a child treated with growth hormone peripubertally enters puberty has been proposed as another alternative to priming (19). Though it is common in practice to continue growth hormone therapy once diagnosed with GHD until the epiphyses close, GH therapy could be paused at onset of puberty to repeat the GH stimulation test and determine if continued therapy is necessary. We recommend obtaining pubertal hormone levels at time of GH stimulation testing to correlate GH response to pubertal status. In our clinical experience, we have cared for patients with low peak GH response to stimulation without priming in whom we elected not to treat that later demonstrated adequate growth velocity and adult height. This demonstrates the importance of the clinician's interpretation of clinical findings in combination with stimulation testing results.

DISCUSSION

Given its flaws, one should enter GH ST with a high predictive value. The Pediatric Endocrine Society recommends against the use of GH stimulation testing as the sole diagnostic criterion of GHD (2). The decision to proceed with growth hormone (GH ST) stimulation testing should be reached only after careful consideration and only when the result will significantly contribute to the diagnostic process (9). If one combines stimulation testing result with the patient's anthropometric measurements, height velocity, physical findings, screening tests, and IGF-1 and IGF-BP3 levels, more complete clinical picture is capture that allows for proper individualized diagnosis and treatment (1). While by itself growth hormone stimulation testing is unreliable, within the overall picture of a patient with short stature, decreased growth velocity, and low IGF-1 level, the results of

growth hormone stimulation testing may complete a picture that the astute clinician can properly utilize to decide on interventions such as growth hormone therapy.

In conclusion we recommend careful, long term observation of patients with growth failure. The decision to undertake growth hormone stimulation should be reserved for those in whom its results would be the last and deciding parameter for therapeutic intervention. If still unclear, additional observation and evaluations such as genetic testing and perhaps repeat stimulation testing should be considered.

AUTHOR CONTRIBUTIONS

MY contributed with substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work, and drafting the work or revising it critically for important intellectual content. RR contributed by providing approval for publication of the content and analysis or interpretation of data for the work, and drafting the work or revising it critically for important intellectual content. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Graber E, Rapaport R. Growth and Growth Disorders in Children and Adolescents. *Pediatr Ann* (2012) 41(4):e1–9. doi: 10.3928/00904481-20120307-07
2. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, et al. Guidelines for Growth Hormone and Insulin-Like Growth Factor-I Treatment in Children and Adolescents: Growth Hormone Deficiency, Idiopathic Short Stature, and Primary Insulin-Like Growth Factor-I Deficiency. *Horm Res Paediatr* (2016) 86(6):361–97. doi: 10.1159/000452150
3. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, et al. Consensus Statement on the Diagnosis and Treatment of Children With Idiopathic Short Stature: A Summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab* (2008) 93(11):4210–7. doi: 10.1210/jc.2008-0509
4. Chesover AD, Dattani MT. Evaluation of Growth Hormone Stimulation Testing in Children. *Clin Endocrinol (Oxf)* (2016) 84(5):708–14. doi: 10.1111/cen.13035
5. Cohen P, Germak J, Rogol AD, Weng W, Kappelgaard AM, Rosenfeld RG, et al. Variable Degree of Growth Hormone (GH) and Insulin-Like Growth Factor (IGF) Sensitivity in Children With Idiopathic Short Stature Compared With GH-Deficient Patients: Evidence From an IGF-Based Dosing Study of Short Children. *J Clin Endocrinol Metab* (2010) 95(5):2089–98. doi: 10.1210/jc.2009-2139
6. van Vught AJ, Nieuwenhuizen AG, Gerver WJ, Veldhorst MA, Brummer RJ, Westerterp-Plantenga MS, et al. Pharmacological and Physiological Growth Hormone Stimulation Tests to Predict Successful GH Therapy in Children. *J Pediatr Endocrinol Metab* (2009) 22(8):679–94. doi: 10.1515/JPEM.2009.22.8.679
7. Gandrud LM, Wilson DM. Is Growth Hormone Stimulation Testing in Children Still Appropriate? *Growth Horm IGF Res* (2004) 14(3):185–94. doi: 10.1016/j.ghir.2003.11.003
8. Cohen P. Statement 1: A Serum Insulin-Like Growth Factor I (IGF-I) Level Should be Part of the Evaluation of Children With Short Stature. *Pediatr Endocrinol Rev* (2008) 5 Suppl 3:834–6.
9. Yau M, Chacko E, Regelmann MO, Annunziato R, Wallach EJ, Chia D, et al. Peak Growth Hormone Response to Combined Stimulation Test in 315 Children and Correlations With Metabolic Parameters. *Horm Res Paediatr* (2019) 92(1):36–44. doi: 10.1159/000502308
10. Muster L, Zangen DH, Nesher R, Hirsch HJ, Muster Z, Gillis D, et al. Arginine and Clonidine Stimulation Tests for Growth Hormone Deficiency Revisited–do We Really Need So Many Samples? *J Pediatr Endocrinol Metab* (2009) 22(3):215–23. doi: 10.1515/JPEM.2009.22.3.215
11. Jaruratanasirikul S, Leethanaporn K, Sriplung H. Should the Duration of the Insulin Tolerance Test be Shortened to 90 Minutes? *J Pediatr Endocrinol Metab* (2004) 17(8):1105–9. doi: 10.1515/JPEM.2004.17.8.1105
12. Galluzzi F, Stagi S, Parpagnoli M, Losi S, Pagnini I, Favelli F, et al. Oral Clonidine Provocative Test in the Diagnosis of Growth Hormone Deficiency in Childhood: Should We Make the Timing Uniform? *Horm Res* (2006) 66(6):285–8. doi: 10.1159/000095781
13. Strich D, Terespolsky N, Gillis D. Glucagon Stimulation Test for Childhood Growth Hormone Deficiency: Timing of the Peak Is Important. *J Pediatr* (2009) 154(3):415–9. doi: 10.1016/j.jpeds.2008.08.044
14. Ribeiro de Oliveira Longo Schweizer J, Ribeiro-Oliveira A Jr, Bidlingmaier M. Growth Hormone: Isoforms, Clinical Aspects and Assays Interference. *Clin Diabetes Endocrinol* (2018) 4:18. doi: 10.1186/s40842-018-0068-1
15. Baumann GP. Growth Hormone Isoforms. *Growth Horm IGF Res* (2009) 19(4):333–40. doi: 10.1016/j.ghir.2009.04.011
16. Clemmons DR. Consensus Statement on the Standardization and Evaluation of Growth Hormone and Insulin-Like Growth Factor Assays. *Clin Chem* (2011) 57(4):555–9. doi: 10.1373/clinchem.2010.150631
17. Bright GM, Do MT, McKew JC, Blum WF, Thorner MO. Development of a Predictive Enrichment Marker for the Oral GH Secretagogue LUM201 in Pediatric Growth Hormone Deficiency. *J Endocrine Soc* (2021) 5(6):bvab030. doi: 10.1210/jendso/bvab030
18. Blum WF, Bright GM, Do MT, McKew JC, Chen H, Thorner MO, et al. Corroboration of Height Velocity Prediction Markers for rhGH With an Oral GH Secretagogue Treatment in Children With GHD. *J Endocrine Soc* (2021) 5(6):bvab029. doi: 10.1210/jendso/bvab029
19. Allen DB. The Diagnosis of Growth Hormone Deficiency Remains a Judgment Call - and That Is Good. *Horm Res Paediatr* (2021) 94:406–9. doi: 10.1159/000521628
20. Wagner IV, et al. Clinical Evidence-Based Cutoff Limits for GH Stimulation Tests in Children With a Backup of Results With Reference to Mass Spectrometry. *Eur J Endocrinol* (2014) 171(3):389–97.
21. Lotierzo M, et al. Comparative Study of Human Growth Hormone Measurements: Impact on Clinical Interpretation. *Clin Chem Lab Med* (2022) 60(2):191–7.
22. Xu C, Zhang X, Dong L, Zhu B, Xin T. MRI Features of Growth Hormone Deficiency in Children With Short Stature Caused by Pituitary Lesions. *Exp Ther Med* (2017) 13(6):3474–8. doi: 10.3892/etm.2017.4377
23. Maghnie M, Salati B, Bianchi S, Rallo M, Tinelli C, Autelli M, et al. Relationship Between the Morphological Evaluation of the Pituitary and the Growth Hormone (GH) Response to GH-Releasing Hormone Plus Arginine in Children and Adults With Congenital Hypopituitarism. *J Clin Endocrinol Metab* (2001) 86(4):1574–9. doi: 10.1210/jcem.86.4.7394
24. Lee HS, Hwang JS. Influence of Body Mass Index on Growth Hormone Responses to Classic Provocative Tests in Children With Short Stature. *Neuroendocrinology* (2011) 93(4):259–64. doi: 10.1159/000326838
25. Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, et al. Effects of Sex and Age on the 24-Hour Profile of Growth Hormone Secretion in Man: Importance of Endogenous Estradiol Concentrations. *J Clin Endocrinol Metab* (1987) 64(1):51–8. doi: 10.1210/jcem-64-1-51
26. Iranmanesh A, Lizarralde G, Veldhuis JD. Age and Relative Adiposity Are Specific Negative Determinants of the Frequency and Amplitude of Growth Hormone (GH) Secretory Bursts and the Half-Life of Endogenous GH in Healthy Men. *J Clin Endocrinol Metab* (1991) 73(5):1081–8. doi: 10.1210/jcem-73-5-1081
27. Makimura H, Feldpausch MN, Rope AM, Hemphill LC, Torriani M, Lee H, et al. Metabolic Effects of a Growth Hormone-Releasing Factor in Obese Subjects With Reduced Growth Hormone Secretion: A Randomized Controlled Trial. *J Clin Endocrinol Metab* (2012) 97(12):4769–79. doi: 10.1210/jc.2012-2794
28. Radack JA, White PC, Adams-Huet B, Oden JD. Stimulated Growth Hormone Concentrations in Obese Pediatric Patients With Mild and Severe Insulin

Resistance: A Pilot Study. *J Pediatr Endocrinol Metab* (2010) 23(4):355–61. doi: 10.1515/jpem.2010.056
29. Qu XD, Gaw Gonzalo IT, Al Sayed MY, Cohan P, Christenson PD, Swerdloff RS, et al. Influence of Body Mass Index and Gender on Growth Hormone (GH) Responses to GH-Releasing Hormone Plus Arginine and Insulin Tolerance Tests. *J Clin Endocrinol Metab* (2005) 90(3):1563–9. doi: 10.1210/jc.2004-1450
30. Stanley TL, Feldpausch MN, Murphy CA, Grinspoon SK, Makimura H. Discordance of IGF-1 and GH Stimulation Testing for Altered GH Secretion in Obesity. *Growth Horm IGF Res* (2014) 24(1):10–5. doi: 10.1016/j.ghir.2013.11.001
31. Loche S, Guzzetti C, Pilia S, Ibba A, Civolani P, Porcu M, et al. Effect of Body Mass Index on the Growth Hormone Response to Clonidine Stimulation Testing in Children With Short Stature. *Clin Endocrinol (Oxf)* (2011) 74 (6):726–31. doi: 10.1111/j.1365-2265.2011.03988.x
32. Rasmussen MH, Hvidberg A, Juul A, Main KM, Gotfredsen A, Skakkebaek NE, et al. Massive Weight Loss Restores 24-Hour Growth Hormone Release Profiles and Serum Insulin-Like Growth Factor-I Levels in Obese Subjects. *J Clin Endocrinol Metab* (1995) 80(4):1407–15. doi: 10.1210/jcem.80.4.7536210
33. Argente J, Caballo N, Barrios V, Muñoz MT, Pozo J, Chowen JA, et al. Multiple Endocrine Abnormalities of the Growth Hormone and Insulin-Like Growth Factor Axis in Patients With Anorexia Nervosa: Effect of Short- and Long-Term Weight Recuperation. *J Clin Endocrinol Metab* (1997) 82 (7):2084–92. doi: 10.1210/jcem.82.7.4090
34. Williams T, Berelowitz M, Joffe SN, Thorner MO, Rivier J, Vale W, et al. Impaired Growth Hormone Responses to Growth Hormone-Releasing Factor in Obesity. A Pituitary Defect Reversed With Weight Reduction. *N Engl J Med* (1984) 311(22):1403–7. doi: 10.1056/NEJM198411293112203
35. Tanaka K, Inoue S, Numata K, Okazaki H, Nakamura S, Takamura Y, et al. Very-Low-Calorie Diet-Induced Weight Reduction Reverses Impaired Growth Hormone Secretion Response to Growth Hormone-Releasing Hormone, Arginine, and L-Dopa in Obesity. *Metabolism* (1990) 39(9):892–6. doi: 10.1016/0026-0495(90)90296-O
36. Luque RM, Kineman RD. Impact of Obesity on the Growth Hormone Axis: Evidence for a Direct Inhibitory Effect of Hyperinsulinemia on Pituitary Function. *Endocrinology* (2006) 147(6):2754–63. doi: 10.1210/en.2005-1549
37. Lee EJ, Kim KR, Lee HC, Cho JH, Nam MS, Nam SY, et al. Acipimox Potentiates Growth Hormone Response to Growth Hormone-Releasing Hormone by Decreasing Serum Free Fatty Acid Levels in Hyperthyroidism. *Metabolism* (1995) 44(11):1509–12. doi: 10.1016/0026-0495(95)90154-X
38. Kok P, Buijs MM, Kok SW, Van Ierssel IH, Frölich M, Roelfsema F, et al. Acipimox Enhances Spontaneous Growth Hormone Secretion in Obese Women. *Am J Physiol Regul Integr Comp Physiol* (2004) 286(4):R693–8. doi: 10.1152/ajpregu.00595.2003
39. Coutant R, Lahlou N, Bouvattier C, Bougnères P. Circulating Leptin Level and Growth Hormone Response to Stimulation Tests in Obese and Normal Children. *Eur J Endocrinol* (1998) 139(6):591–7. doi: 10.1530/eje.0.1390591
40. Martha PM Jr, Gorman KM, Blizzard RM, Rogol AD, Veldhuis JD. Endogenous Growth Hormone Secretion and Clearance Rates in Normal Boys, as Determined by Deconvolution Analysis: Relationship to Age, Pubertal Status, and Body Mass. *J Clin Endocrinol Metab* (1992) 74(2):336–44. doi: 10.1210/jcem.74.2.1730812
41. Leung KC, Johannsson G, Leong GM, Ho KKY. Estrogen Regulation of Growth Hormone Action. *Endocr Rev* (2004) 25(5):693–721. doi: 10.1210/er.2003-0035
42. Growth Hormone Research S. Consensus Guidelines for the Diagnosis and Treatment of Growth Hormone (GH) Deficiency in Childhood and Adolescence: Summary Statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab* (2000) 85(11):3990–3. doi: 10.1210/jcem.85.11.6984
43. Murray PG, Dattani MT, Clayton PE. Controversies in the Diagnosis and Management of Growth Hormone Deficiency in Childhood and Adolescence. *Arch Dis Child* (2016) 101(1):96–100. doi: 10.1136/archdischild-2014-307228
44. Sato T, Kusakawa M, Ichihashi Y, Ishii T, Hasegawa T. Testosterone Priming Increased Growth Hormone Peak Levels in the Stimulation Test and Suppressed Gonadotropin Secretion in Three Japanese Adolescent Boys. *Clin Pediatr Endocrinol* (2020) 29(3):119–21. doi: 10.1297/cpe.29.119
45. Marin G, Domené HM, Barnes KM, Blackwell BJ, Cassorla FG, Cutler GB Jr, et al. The Effects of Estrogen Priming and Puberty on the Growth Hormone Response to Standardized Treadmill Exercise and Arginine-Insulin in Normal Girls and Boys. *J Clin Endocrinol Metab* (1994) 79(2):537–41. doi: 10.1210/jcem.79.2.8045974
46. Müller G, Keller A, Reich A, Hoepffner W, Kratzsch J, Buckler JM, et al. Priming With Testosterone Enhances Stimulated Growth Hormone Secretion in Boys With Delayed Puberty. *J Pediatr Endocrinol Metab* (2004) 17(1):77–83. doi: 10.1515/JPEM.2004.17.1.77
47. Hage C, Gan HW, Ibba A, Patti G, Dattani M, Loche S, et al. Advances in Differential Diagnosis and Management of Growth Hormone Deficiency in Children. *Nat Rev Endocrinol* (2021) 17(10):608–24. doi: 10.1038/s41574-021-00539-5
48. Binder G, Reinehr T, Ibáñez L, Thiele S, Linglart A, Woelfle J, et al. GHD Diagnostics in Europe and the US: An Audit of National Guidelines and Practice. *Horm Res Paediatr* (2019) 92(3):150–6. doi: 10.1159/000503783
49. Lazar L, Phillip M. Is Sex Hormone Priming in Peripubertal Children Prior to Growth Hormone Stimulation Tests Still Appropriate? *Horm Res Paediatr* (2010) 73(4):299–302. doi: 10.1159/000284396
50. Albrecht A, Penger T, Marx M, Hirsch K, Dörr HG. Short-Term Adverse Effects of Testosterone Used for Priming in Prepubertal Boys Before Growth Hormone Stimulation Test. *J Pediatr Endocrinol Metab* (2018) 31(1):21–4. doi: 10.1515/jpem-2017-0280
51. Rose SR, Municchi G, Barnes KM, Kamp GA, Uriarte MM, Ross JL, et al. Spontaneous Growth Hormone Secretion Increases During Puberty in Normal Girls and Boys. *J Clin Endocrinol Metab* (1991) 73(2):428–35. doi: 10.1210/jcem-73-2-428

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Endocrinology

TYPE Mini Review
PUBLISHED 22 August 2022
DOI 10.3389/fendo.2022.980979

OPEN ACCESS

EDITED BY
Sandro Loche,
Ospedale Microcitemico, Italy

REVIEWED BY
Giorgio Radetti,
Ospedale di Bolzano, Italy
Susan R. Rose,
Cincinnati Children's Hospital Medical Center, United States

*CORRESPONDENCE
Bradley S. Miller
mille685@umn.edu

SPECIALTY SECTION
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 29 June 2022
ACCEPTED 04 August 2022
PUBLISHED 22 August 2022

CITATION
Miller BS (2022) What do we do now that the long-acting growth hormone is here?.
Front. Endocrinol. 13:980979.
doi: 10.3389/fendo.2022.980979

What do we do now that the long-acting growth hormone is here?

Bradley S. Miller*

Pediatric Endocrinology, University of Minnesota Medical School, Minneapolis, MN, United States

In standard 52-week phase III clinical trials, once weekly lonapegsomatropin, somatrogon and somapacitan have been found to yield non-inferior height velocities and similar safety profiles to daily GH (DGH) in children with pediatric growth hormone deficiency (PGHD).

Lonapegsomatropin, a long-acting GH therapy (LAGH), was approved by the United States Food and Drug Administration (FDA) in August 2021 for the treatment of PGHD and has also been approved in other regions of the world. Somatrogon was approved for the treatment of PGHD beginning in some regions beginning in late 2021. Somapacitan was approved by the FDA for the treatment of Adult GHD in August 2020. The phase III clinical trial of somapacitan for the treatment of PGHD has been completed and demonstrated non-inferiority of somapacitan to DGH.

New LAGH products may improve patient adherence, quality of life and clinical outcomes, particularly in patients with poor adherence to daily GH injections in the future. With the availability of new LAGH products, clinicians will need to identify the best candidates for LAGH therapy and understand how to monitor and adjust therapy. Long-term surveillance studies are needed to demonstrate adherence, efficacy, cost-effectiveness and safety of LAGH preparations and to understand how the non-physiological pharmacokinetic and pharmacodynamic profiles following administration of each LAGH product relate to short- and long-term safety and efficacy of LAGH therapy.

KEYWORDS

long-acting growth hormone, pediatric growth hormone deficiency, lonapegsomatropin, somapacitan, somatrogon

Introduction

This article describes the rationale for using long-acting GH therapy (LAGH), previous attempts at generating LAGH preparations by different pharmaceutical companies, LAGH therapies currently in development or approved around the world, insulin-like growth factor I (IGF-I) monitoring during LAGH therapy, patient selection for LAGH therapy and the future of LAGH.

Daily recombinant human GH (DGH) therapy became available for the treatment of pediatric GHD (PGHD) in 1985 and adult GHD (AGHD) in 1996. However, because of the need for daily injections, individual adherence to GH has been shown to decrease over time with concomitant reductions in height velocity and IGF-I levels in the short term in children and adolescents. In a recent analysis of electronic medical records following initiation of DGH, adherent patients gained an additional 1.8 cm over 1 year compared to non-adherent patients (1). It is likely that reduced adherence to daily injections limits treatment outcomes as evidenced by adult height in children who required GH replacement therapy that are below the mean for the population (2–8). Thus, it has been hypothesized that LAGH products might help mitigate treatment non-adherence and potentially improve long term treatment effects in patients with PGHD.

Status of long-acting growth hormone products

Nutropin Depot®, rhGH released slowly from biodegradable microspheres, was the first LAGH approved for PGHD in 1999, but was removed from the market in 2004 due to marketing and manufacturing issues. Since then, a number of other attempts have been made to develop LAGH products using different approaches to prolong the half-life of the GH molecule (9), including unmodified rhGH in a depot formulation (i.e. Eutropin Plus®), pegylated rhGH (i.e. Jintrolong®), modification of rhGH to increase albumin binding (i.e. somapacitan, Sogroya®), rhGH fusion proteins (i.e. somatrogon, NGENLA®) and prodrug releasing unmodified rhGH (i.e. lonapegsomatropin, Skytrofa®). Eutropin Plus® (South Korea), Jintrolong® (China), Skytrofa® (lonapegsomatropin; US) and NGENLA® (somatrogon; European Union, Canada, Australia and Japan) are currently approved and available for treatment of PGHD (Table 1). Somapacitan (Sogroya®; US, EU, Japan) is currently approved for AGHD but not PGHD.

Lonapegsomatropin

Lonapegsomatropin is a prodrug consisting of unmodified GH transiently conjugated to methoxypolyethylene glycol (Table 1). This transient chemical modification allows time-release of unmodified GH with a half-life of ~25 hours allowing for once-weekly administration. Clinical trials of lonapegsomatropin have demonstrated positive efficacy results in children (Phase 2 and 3) and adults (Phase 2) with GHD. In the phase III trial of lonapegsomatropin in PGHD, children receiving lonapegsomatropin 0.24 mg/kg once weekly grew 11.2 cm/yr compared to 10.3 cm/yr for children receiving DGH at a dose of 0.24 mg/kg/wk. The estimated treatment difference was 0.86 cm (95% Confidence intervals, 0.22 to 1.50) demonstrating non-inferiority and statistical superiority of lonapegsomatropin compared to DGH (Table 2, Figure 1) (10). No concerning side effects have been demonstrated with lonapegsomatropin in children or adults.

Children receiving lonapegsomatropin in the extension portions of the phase III trials have continued to show efficacy and safety (13–15). After completion of the 12 month pivotal randomized trial comparing lonapegsomatropin to DGH (heiGHt) or completion of the 6 month switch trial (fliGHt), children were able to enroll in the enliGHten extension study. In the most recently available data for the enliGHten trial, at 130 weeks children had reached an average height SDS of -0.64 compared to their midparental target height SDS of -0.39. In the 36 children who completed the trial, they had reached an average height SDS of -0.38 with a difference compared to their mid-parental target height SDS of -0.05 (15).

In the phase III heiGHt trial of lonapegsomatropin in PGHD, children receiving lonapegsomatropin 0.24 mg/kg once weekly had mean IGF-I SDS at week 52 of +0.72 SDS compared to -0.02 SDS in children receiving DGH (Table 2). Two children

TABLE 1 Long-acting growth hormone characteristics and approval locations.

	Lonapegsomatropin	Somatrogon	Somapacitan
Mechanism	Reversible Pegylation	Fusion Protein with hCG CTP (x3)	Acylation increases reversible binding to endogenous Albumin
Molecular Weight of Active Agent (kDa)	22	41	23.3
Approval	US, EU	EU, Canada, Australia, Japan	US, EU, Japan (AGHD only)

hCG, human chorionic gonadotropin; CTP, c-terminal peptide; kDa, kilodalton; US, United States; EU, European Union; AGHD, adult growth hormone deficiency.

TABLE 2 Long-acting growth hormone treatment response at 52 weeks.

	Lonapegsomatropin (10)	Somatrogon (11)	Somapacitan (12)
Dose (mg/kg/wk)	0.24	0.66	0.16
Height Velocity LAGH (cm/yr)	11.2	10.1	11.2
Height Velocity DGH (cm/yr) (0.24 mg/kg/wk)	10.3	9.8	11.7
Estimated Treatment Difference (cm/yr), (95% CI)	0.9 (0.2-1.5)	0.3 (-0.2, 0.9)	-0.5 (-1.1, 0.2)
Estimated Average IGF-I SDS	+0.72	+0.65	+0.28
IGF-I SDS DGH	-0.02	-0.69	+0.10

mg, milligram; kg, kilogram; wk, week; cm, centimeter; yr, year; DGH, daily growth hormone; CI, confidence interval; IGF-I, insulin-like growth factor I; SDS, standard deviation score.

had dose reductions due to asymptomatic elevations of IGF-I (10). In the phase III fliGHt switch trial, the average IGF-I obtained five days after lonapegsomatropin injection was +1.6 SDS. For subjects who had IGF-I values ≤2 SDS at entry into the fliGHt trial (already receiving DGH therapy), 31.2% of children had IGF-I values five days after lonapegsomatropin injection > +2 SDS at 26 weeks (14). In the most recently available data for the enliGHten trial, at 130 weeks children had an average IGF-I value five days after lonapegsomatropin injection of +1.46 SDS (15). Dose reductions of lonapegsomatropin occurred in 29.9% of children in the trial resulting in an average lonapegsomatropin dose of 0.212 mg/kg/wk (13, 15).

Somatrogon

Somatrogon consists of GH with the addition of three cassettes representing the c-terminal peptide (CTP) of human chorionic gonadotropin resulting in a fusion protein of approximately 41 kDa (Table 1). The addition of the CTP cassettes gives somatrogon a prolonged *in vivo* half-life in comparison with native GH allowing for once-weekly administration of somatrogon.

The phase III trial of somatrogon in AGHD (ClinicalTrials.gov Identifier: NCT01909479) was completed in 2016 and failed to meet the primary endpoint (16). However, the phase III trial of somatrogon in PGHD (ClinicalTrials.gov Identifier: NCT02968004) that was completed in 2019 demonstrated non-inferiority of somatrogon to DGH (17, 18). Based upon these results, somatrogon received market authorization for treatment of PGHD in the European Union by the European Medicines Agency in February 2022 (19). Additionally, somatrogon is approved for PGHD in Australia, Canada and Japan and is marketed as NGENLA® (Table 1). A Biologics License Application for somatrogon for the treatment of PGHD was submitted to the US Food and Drug

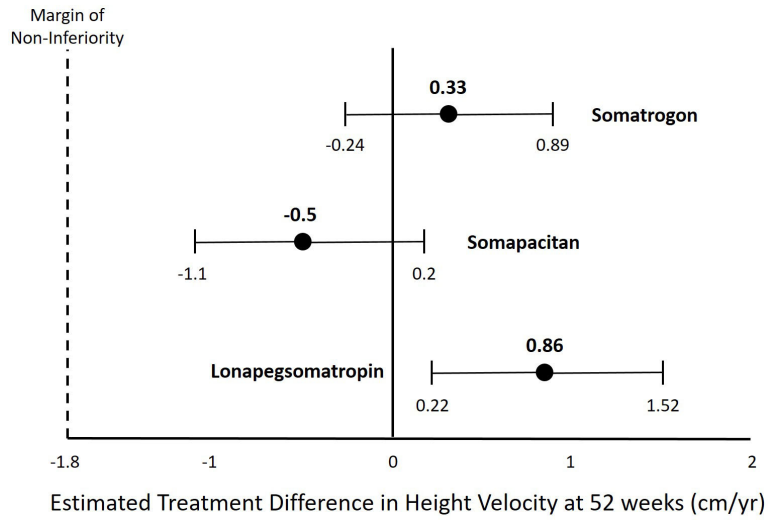


FIGURE 1
Estimated Treatment Difference in Height Velocity for Different Long-Acting Growth Hormone Products.

Administration (FDA) in 2021 and received a Complete Response Letter in January 2022, but is currently not approved in the US (20). In the phase III trial of somatrogon in PGHD, children receiving somatrogon 0.66 mg/kg once weekly grew 10.1 cm/yr compared to 9.8 cm/yr for children receiving DGH at a dose of 0.24 mg/kg/wk. The estimated treatment difference was 0.33 cm (95% confidence interval, -0.24 to 0.89) demonstrating non-inferiority of somatrogon compared to DGH (Table 2, Figure 1). No concerning side effects have been demonstrated with somatrogon in children or adults. Anti-drug antibodies have been reported in a significant number of children receiving somatrogon. However, there has not been any demonstration that the reported anti-drug antibodies have been neutralizing or had a negative impact on the growth of children receiving somatrogon. In a report of long-term growth with up to 4 years somatrogon therapy in PGHD from the extension portions of the phase II and III trials, the achieved height velocities and height z-scores were similar or slightly better than expected compared to historical controls from the Pfizer registry KIGS (21).

In the phase III trial of somatrogon in PGHD, children receiving somatrogon 0.66 mg/kg once weekly had mean IGF-I SDS at week 52 of +0.65 SDS compared to -0.69 SDS in children receiving DGH (Table 2) (11). There are no data available yet for IGF-I values in children treated with somatrogon for longer periods.

Somapacitan

Somapacitan consists of GH with one amino acid substitution in an area not involved in GH receptor binding (Table 1). An acyl linker that functions as an albumin binding moiety is covalently attached to the modified amino acid. The albumin binding moiety reversibly binds to endogenous albumin gives somapacitan a prolonged *in vivo* half-life in comparison with native GH allowing for once-weekly administration of somapacitan.

Sogroya® (somapacitan; US, Europe, Japan) was approved by the FDA for treatment of AGHD in August 2020, but is yet to be commercially available in the US. It is available in the EU and Japan (Table 1). The somapacitan phase III trial in PGHD (ClinicalTrials.gov Identifier: NCT03811535) was completed in 2021 and the results were reported recently, but are not yet published in the peer-reviewed literature (22, 23). In the phase III trial of somapacitan in PGHD, children receiving somapacitan 0.16 mg/kg once weekly grew 11.2 cm/yr compared to 11.7 cm/yr for children receiving DGH at a dose of 0.24 mg/kg/wk. The estimated treatment difference was -0.5 cm (95% confidence interval, -1.1 to 0.2) demonstrating non-inferiority of somapacitan compared to DGH (Table 2, Figure 1) (12). In a report of long-term growth with up to 4 years somapacitan therapy in PGHD from the extension portions of the phase II trial (REAL3), there was a mean height SDS change from baseline of 2.85 SDS for those who received somapacitan throughout and 2.28 SDS for those who received DGH for two years before switching to weekly somapacitan (24). With a baseline height SDS for the somapacitan groups of approximately -3.8 SDS, the height SDS after 4 years somapacitan therapy would be approximately -0.95 SDS. With a baseline height SDS of -3.4 for the group who received DGH before switching to somapacitan the height SDS after 4 years somapacitan therapy would be -1.12 SDS (25). No concerning side effects have been demonstrated with somapacitan in children or adults.

In the phase III trial of somapacitan in PGHD, children receiving somapacitan 0.16 mg/kg once weekly had mean IGF-I SDS at week 52 of +0.28 SDS compared to +0.10 SDS in children receiving DGH (Table 2) (12). In the extension studies of somapacitan, mean IGF-I SDS at year 4 was +1.29 SDS for those who received somapacitan throughout and +0.94 SDS for those who received DGH for two years before switching to weekly somapacitan (24).

IGF-I monitoring

The pharmacodynamics of the different LAGH products have been measured using IGF-I as the biomarker. Based upon the pharmacodynamic models, the peak IGF-I levels occur between 2 and 3 days and the average IGF-I level occurs between four and six days. Using IGF-I data from the phase 2 and phase 3 clinical trials in children, pharmacodynamic models have been developed to estimate the average IGF-I concentrations from a single serum sample obtained at any time after an injection of LAGH at study state (26–28). It is necessary that the timing of the injection and the timing of collection of the serum sample are known in order to calculate the estimated average IGF-I level. Therefore, if a convenience sample is obtained four to five days after the injection, it is a reasonable estimate of the average IGF-I. If the sample is collected at any other time following the injection, an IGF-I calculator can be used to calculate the estimated average IGF-I. An IGF-I calculator is available for lonapegsomatropin (27). The shape of the pharmacodynamic curve should be identical regardless of the method of IGF-I assay. Therefore, the IGF-I calculator could be very useful regardless of the type of IGF-I assay used. However, these calculators and pharmacodynamic models need to be evaluated further in a broader population of children with GHD, including pubertal children and transition patients. Additionally, since IGF-I values are not normally distributed, the SDS values may vary by age and assay (29).

For this reason, further validation of these models with different IGF-I assays is needed.

Dose adjustment

LAGH has been studied in clinical trials using a weight-based dosing paradigm (11, 12, 15). In this dosing paradigm, similar to DGH, the dose of LAGH was adjusted for the weight of the child at specified clinical research visits. In the published data, the only other dose adjustments occurred were due to elevated IGF-I levels or adverse events. Therefore, there is little information available to guide the clinician on how to adjust the dose of the different LAGH products. In clinical practice, adjustment of DGH dosing has been based upon weight or body surface area, height velocity and/or IGF-I levels. The use of IGF-I levels to guide dose adjustment of DGH therapy has been recommended for both safety and short-term efficacy purposes. From a safety perspective, it has been recommended that GH therapy increases IGF-I levels to rise into the normal range (i.e. ≤ +2 SDS) (30, 31). From an efficacy perspective, targeting an IGF-I in the upper part of the normal range (+1 to +2 SDS) has been suggested in order to improve short-term efficacy (32). However, long-term efficacy of this approach has not been demonstrated. Weight-based dosing of DGH has been shown to achieve an IGF-I level close to 0 SDS depending upon the dose used (33).

As described earlier, the pharmacodynamic profiles of IGF-I levels following an injection of the different LAGH products show a significant increase of IGF-I from baseline to peak and with return to baseline before the next injection (11, 12, 15). It is likely that the efficacy of LAGH will correlate with the average IGF-I level achieved (32). Therefore, it will be important to be able to estimate an average IGF-I from serum samples collected at random clinic visits. Using the IGF-I calculator to estimate average IGF-I values from these samples may help guide dose adjustment of LAGH (11, 12, 15). Lonapegsomatropin, somatrogon and somapacitan have been shown to have a linear IGF-I dose response during clinical trials suggesting that predictable changes in average IGF-I levels can be achieved with adjustments in dose of these LAGH molecules (15, 28, 34, 35).

There have been concerns raised that short-term elevations of GH and IGF-I during LAGH therapy may be associated with short- and long-term adverse events (29, 31). The GH Research Society consensus guidelines suggested that the goal of LAGH therapy is to maintain IGF-I levels within the age-appropriate range for the majority of the treatment period, as IGF-I levels maintained within such age-appropriate range correlates with safety of treatment (31). However, peak IGF-I levels, using the pharmacodynamic model, may be able to be estimated for future analysis of their relationship to safety and efficacy. Clinicians interested in measuring peak IGF-I levels following LAGH administration at steady-state could obtain IGF-I measurements between 2 and 3 days after an injection (26–28).

Patient selection for LAGH

When selecting children with GHD for treatment with LAGH, providers may consider a number of different characteristics known to negatively impact adherence. Potential candidates for LAGH include individuals with poor adherence, particularly teenagers, young children expected to be on therapy for many years, children with needle phobia, children transitioning to self-injection and patients on multiple other medications, particularly injectable medications like insulin. Good candidates will likely be a highly motivated subset of this list of potential candidates. The prescriber needs to recognize that children with poor adherence with DGH may still have poor adherence with LAGH. Based upon the short-term efficacy and safety data, providers are also likely to start LAGH in naïve children.

Although lonapegsomatropin is approved for PGHD down to 1 year of age and somatrogon is approved for PGHD down to 3 years of age, children with severe GHD associated with hypoglycemia may not be good candidates for LAGH products since they may be at increased risk of hypoglycemia at trough GH levels occurring in the day or two prior to each injection. Since hypoglycemia can occur in children above 3 years of age in isolated PGHD or PGHD associated with multiple pituitary hormone deficiencies, it will be important for providers to recognize this potential risk when considering LAGH therapy. Glucose measurements have been collected in children with PGHD during clinical trials of lonapegsomatropin, somatrogon and somapacitan without any reports of hypoglycemia. However, children with hypoglycemia associated with PGHD would not be naïve at the ages recruited into those clinical trials. Therefore, the occurrence of hypoglycemia in PGHD needs to be studied carefully and may warrant a different dosing paradigm of LAGH products, such as twice weekly injections instead of weekly.

Cancer survivors with PGHD are a group of children who warrant careful thought when considering LAGH therapy. DGH has not been shown to cause recurrent neoplasms, but concern about a small increased risk for subsequent neoplasms overall in pediatric cancer survivors remains (36). Therefore, theoretical concerns about transient elevations of GH and IGF-I that occur with each LAGH dose may lead providers to hesitate when considering LAGH therapy in cancer survivors with PGHD (37). Alternatively, providers may select an LAGH product that has a more flat IGF-I profile with fewer IGF-I excursions above +2 or +3 SDS, a lower LAGH dose or both. If clinicians diagnose PGHD early in cancer survivors, GH replacement therapy may be initiated before the height percentile declines below the normal range, and maintenance of a normal growth rate is sufficient. Thus, use of a higher GH dose to achieve catch-up growth would not be necessary. As more safety and efficacy data for LAGH emerges and as experience with LAGH products

grows, it is possible that LAGH may potentially replace DGH in the treatment of PGHD.

Early experience with LAGH

Since the approval of Skytrofa® (lonapegsomatropin) for PGHD in the US in August 2021, numerous pediatric endocrinologists have begun to prescribe lonapegsomatropin in children. In my personal experience, some children and their families have been reluctant to start lonapegsomatropin instead of DGH or to switch from DGH to lonapegsomatropin. In my practice, the most common reason for families to prefer not to switch to lonapegsomatropin was due to concerns about insurance coverage. In addition to concerns about whether insurance would cover the new product, families were concerned that seeking approval of lonapegsomatropin could lead to a lack of continued approval or renewal of their currently approved DGH. Although this perception may be unique to the US insurance market, it was a powerful deterrent for many families to consider switching to LAGH. It is likely that concern regarding insurance coverage will improve as contracts with insurance providers are developed. FDA approval of other LAGH products may also improve insurance coverage. If adherence and outcomes are demonstrated to be superior in children receiving LAGH, this should also improve insurance coverage of these products. Finally, some children, their families and their providers may be reluctant to transition to LAGH products due to comfort and good growth outcomes with DGH, availability of decades of safety and efficacy data for DGH and fear of change. The cost and cost-effectiveness of LAGH products are also likely to impact treatment decisions. Collection of longitudinal safety and efficacy data for LAGH should help providers provide appropriate reassurance for families interested in treatment with LAGH in the future.

Future directions

The LAGH products in development have been studied primarily in PGHD. Studies to demonstrate safety and efficacy of LAGH for children with other growth disorders are also needed. A phase II study of Eutropin Plus® (LB03002) in idiopathic short stature (ISS) in South Korea showed evidence of non-inferiority of two doses compared to DGH at 0.37 mg/kg/wk (38). A phase II study of 0.16, 0.20 and 0.24 mg/kg/wk somapacitan (REAL5) in short children born Small for Gestational Age (SGA) demonstrated improved height velocity to 8.9, 11.1 and 11.2 cm/yr, respectively. Children with SGA receiving 0.35 and 0.67 mg/kg/wk DGH demonstrated a height velocity of 10.5 and 11.9 cm/yr, respectively. A phase III clinical trial of somapacitan in short children with SGA, Turner Syndrome, Noonan Syndrome and Idiopathic Short Stature (REAL8) is estimated to start in July 2022 (39).

Although a transition from daily injections to once weekly injections has been shown to improve adherence in other treatment areas, it has not yet been demonstrated in children receiving LAGH products (40–43). It will be important to evaluate adherence through standard methodologies including pharmacy refill data. Novel approaches for capturing adherence such as the Bluetooth capability of the electronic injection device for lonapegsomatropin, if approved, will provide additional information to correlate adherence with treatment outcomes. Although LAGH preparations are being evaluated through a regulatory process that requires demonstration of non-inferiority to DGH injections, it is likely that these compounds will result in improved long-term efficacy as well as convenience for patients and their caregivers. This improvement in outcomes will likely be due to the underestimated impact of reduced adherence and persistence with GH therapy. The data from extension studies of lonapegsomatropin, somatrogon and somapacitan demonstrate the potential for LAGH to close the efficacy gap seen with DGH therapy. Long-term studies, including real world evidence, are still needed to demonstrate these benefits as they are crucial in determining the cost-effectiveness and safety of LAGH preparations.

The long-term safety of LAGH products requires further study as they do not mimic the physiologic profile of endogenous GH secretion or the non-physiologic profile of DGH for which we have extensive safety data. It remains to be seen whether this difference in pharmacokinetic and pharmacodynamic profile will exert a positive or negative impact on short- and long-term safety and efficacy. Following the approval of rhGH in 1985, numerous phase 4 post marketing surveillance registries have collected safety and efficacy data for DGH therapy (44). These studies accumulated more than 600,000 patient years of safety and efficacy data and helped our community learn about common and rare side effects of DGH therapy. One of the challenges of these studies was that children were no longer followed after DGH therapy was discontinued, and were therefore lost to follow-up. In order to collect important data regarding linear growth and metabolic outcomes in children receiving LAGH preparations, including lonapegsomatropin, it will be crucial to perform similar phase IV post marketing surveillance studies. However, in order to avoid losing the patients when they complete therapy or transition to another GH product and to capture patient reported outcomes, it is imperative to develop studies utilizing LAGH therapy that follow children long-term well into their adulthood.

Efforts to develop an independent international study to achieve these outcomes is currently underway (GloBE-Reg LAGH (https://globe-reg.net/)) spearheaded by a consortium of pediatric endocrinology societies. These efforts require support from the manufacturers of DGH and LAGH preparations, as well as from the pediatric endocrinology

community. Pfizer has also begun a registry (PROGRES, Pfizer Registry of Outcomes in Growth hormone RESearch) to collect safety and efficacy data in children receiving somatrogon and DGH (45). If registries developed by manufacturers of LAGH products can interact with each other and the GloBE-Reg LAGH study to share data that will increase the power to identify important outcomes. LAGH phase IV registry studies may be useful in validating IGF-I pharmacodynamic models for each LAGH product and in determining the relationship between estimated peak and estimated average IGF-I levels to short- and long-term safety and efficacy of LAGH therapy.

Conclusion

Numerous LAGH preparations have been or are currently being developed, but they each have their unique molecular characteristics and clinical efficacies (9). In standard 52 week phase III clinical trials, once weekly lonapegsomatropin, somatrogon and somapacitan have been found to yield non-inferior height velocities in children with PGHD with safety profiles comparable to DGH. In longer term extension studies, once weekly lonapegsomatropin, somatrogon and somapacitan have been found to have sustained efficacy in children with PGHD. Thus, LAGH preparations have the potential to close the efficacy gap in DGH by reaching a near adult height appropriate for the mid-parental target height and the population. However, it remains to be seen whether these effects can be replicated in real world use of LAGH. LAGH may improve patient adherence, quality of life and clinical outcomes, particularly in patients with poor adherence to DGH injections. Long-term surveillance studies are needed to demonstrate adherence, efficacy, cost-effectiveness and safety of LAGH preparations and to understand the relationship between estimated peak and estimated average IGF-I levels at steady state to short- and long-term safety and efficacy of LAGH therapy.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

Conflict of interest

Dr. Miller is a consultant for AbbVie, Ascendis Pharma, BioMarin, Bristol Myers Squibb, EMD Serono, Endo Pharmaceuticals, Novo Nordisk, Orchard Therapeutics, Pfizer, Tolmar and Vertice and has received research support from Alexion, AbbVie, Aeterna Zentaris, Amgen, Amicus, Lumos Pharma, Lysogene, Novo Nordisk, OPKO Health Pfizer, Prevail Therapeutics and Sangamo Therapeutics.

Publisher's note

References

1. Loftus J, Miller BS, Parzynski CS, Alvir J, Chen Y, Jhingran P, et al. Association of daily growth hormone injection adherence and height among children with growth hormone deficiency. *Endocrine Pract* (2022) 28(6):565–71. doi: 10.1016/j.eprac.2022.02.013

2. August GP, Julius JR, Blethen SL. Adult height in children with growth hormone deficiency who are treated with biosynthetic growth hormone: the national cooperative growth study experience. *Pediatrics* (1998) 102(2 Pt 3):512–6. doi: 10.1542/peds.102.S3.512

3. Bell JJ, Lippe B, Romano AA, Cernich JT, Swinford RD, Moawad D. National cooperative growth study: 25 years of growth hormone data, insights, and lessons for future registries. *Pediatr Endocrinol Rev* (2018) 16(2):240–55. doi: 10.17458/per.vol16.2018.25yearsghdata

4. Child CJ, Zimmermann AG, Chrousos GP, Cummings E, Deal CL, Hasegawa T, et al. Safety outcomes during pediatric GH therapy: Final results from the prospective GeNeSIS observational program. *J Clin Endocrinol Metab* (2019) 104 (2):379–89. doi: 10.1210/jc.2018-01189

5. Pfaffle R, Bidlingmaier M, Kreitschmann-Andermahr I, Land C, Partsch CJ, Schwab KO, et al. Safety and effectiveness of omnitrope r, a biosimilar recombinant human growth hormone: More than 10 years' experience from the PATRO children study. *Hormone Res Paediatrics* (2020) 93(3):154–63. doi: 10.1159/000508190

6. Reiter EO, Price DA, Wilton P, Albertsson-Wikland K, Ranke MB. Effect of growth hormone (GH) treatment on the near-final height of 1258 patients with idiopathic GH deficiency: analysis of a large international database. *J Clin Endocrinol Metab* (2006) 91(6):2047–54. doi: 10.1210/jc.2005-2284

7. Ross JL, Lee PA, Gut R, Germak J. Attaining genetic height potential: Analysis of height outcomes from the ANSWER program in children treated with growth hormone over 5 years. *Growth Hormone Igf Res* (2015) 25(6):286–93. doi: 10.1016/j.ghir.2015.08.006

8. Miller BS, Rose SR, Ross JL, Lee PA, Smith C, Henschel T, et al. Growth hormone dosage associated with change in height standard deviation score over 4 years of treatment by age in pediatric patients with isolated growth hormone deficiency (IGHD). In: *Results from the ANSWER program*. San Diego, CA: Pediatric Academic Societies' Meeting (2015).

9. Miller BS, Velazquez E, Yuen KCJ. Long-acting growth hormone preparations - current status and future considerations. *J Clin Endocrinol Metab* (2020) 105(6):01. doi: 10.1210/clinem/dgz149

10. Thornton PS, Maniatis AK, Aghajanova E, Chertok E, Vlachopapadopoulou E, Lin Z, et al. Weekly lonapegsomatropin in treatment-naive children with growth hormone deficiency: The phase 3 heiGHt trial. *J Clin Endocrinol Metab* (2021) 106 (11):3184–95. doi: 10.1210/clinem/dgab529

11. Deal CL, Steelman J, Vlachopapadopoulou E, Stawerska R, Silverman LA, Phillip M, et al. Efficacy and safety of weekly somatrogon vs daily somatropin in children with growth hormone deficiency: A phase 3 study. *J Clin Endocrinol Metab* (2022) 107(7):e2717–28. doi: 10.1210/clinem/dgac220

12. Miller BS, Blair JC, Hojby M, Böttcher MV, Kildemoes RJ, Maniatis A, et al. *Once-weekly somapacitan is effective and well tolerated in children with GHD: a randomized phase 3 trial*. Atlanta, Georgia, USA: ENDO (2022).

13. Maniatis AK, Casella SJ, Nadgir UM, Hofman PL, Saenger P, Chertock ED, et al. Safety and efficacy of lonapegsomatropin in children with growth hormone deficiency: enliGHten trial 2-year results. *J Clin Endocrinol Metab* (2022) 107(7): e2680–9. doi: 10.1210/clinem/dgac217

14. Maniatis AK, Nadgir U, Saenger P, Reifschneider KL, Abuzzahab J, Deeb L, et al. Switching to weekly lonapegsomatropin from daily somatropin in children with growth hormone deficiency: The fliGHt trial. *Hormone Res Paediatrics* (2022) 09:09. doi: 10.1159/000524003

15. Thornton PS, Nadgir UM, Hofman P, Saenger P, Chertok E, D., Aghajanova EM, et al. *Safety and efficacy of treatment with lonapegsomatropin in children with growth hormone deficiency at week 130 in the enliGHten trial*. Atlanta, Georgia, USA: ENDO (2022).

16. Strasburger CJ, Vanuga P, Payer J, Pfeifer M, Popovic V, Bajnok L, et al. MOD-4023, a long-acting carboxy-terminal peptide-modified human growth hormone: results of a phase 2 study in growth hormone-deficient adults. *Eur J Endocrinol* (2017) 176(3):283–94. doi: 10.1530/EJE-16-0748

17. Deal CL, Pastrak A, Silverman LA, Valluri SR, Wajnrajch MP, Cara JF. OR10-06 somatrogon growth hormone in the treatment of pediatric growth hormone deficiency: Results of the pivotal pediatric phase 3 clinical trial. *J Endocrine Soc* (2020) 4(Supplement_1):A648–9. doi: 10.1210/jendso/bvaa046.1279

18. Pfizer. *OPKO and pfizer announce positive phase 3 top-line results for somatrogon, an investigational long-acting human growth hormone to treat children with growth hormone deficiency* (2019). Available at: https://www.pfizer.com/news/press-release/press-release-detail/opko_and_pfizer_announce_positive_phase_3_top_line_results_for_somatrogon_an_investigational_long_acting_human_growth_hormone_to_treat_children_with_growth_hormone_deficiency. (Accessed 2/24/2022).

19. Pfizer. *Pfizer and OPKO's once-weekly NGENLA™ (somatrogon) injection receives marketing authorization in European union for treatment of pediatric growth hormone deficiency* (2022). Available at: https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-opkos-once-weekly-ngenlatm-somatrogon-injection. (Accessed 2/26/2022).

20. Park B. *FDA Denies approval of long-acting pediatric growth hormone therapy* (2021). Available at: https://www.empr.com/home/news/drugs-in-the-pipeline/fda-denies-approval-of-long-acting-pediatric-growth-hormone-therapy/. (Accessed 2/24/2022).

21. Zadik Z, Cara J, Carlsson M, Wajnrajch MP, Wang R, Rosenfeld RG. Growth outcomes from the phase 2 and phase 3 studies of once weekly somatrogon vs daily genotropin in pediatric patients with growth hormone deficiency. In: *Comparisons with published literature and an international growth study database*. Atlanta, Georgia, USA: ENDO (2022).

22. ClinicalTrials.gov. *A research study in children born small and who stayed small. treatment is somapacitan once a week compared to norditropin® once a day* (2019). Available at: https://clinicaltrials.gov/ct2/show/NCT03878446 (Accessed 2/24/2022).

23. ClinicalTrials.gov. *A research study in children with a low level of hormone to grow. treatment is somapacitan once a week compared to norditropin® once a day (REAL4)* (2019). Available at: https://clinicaltrials.gov/ct2/show/NCT03811535 (Accessed 2/24/2022).

24. Savendahl L, Battelino T, Hojby Rasmussen M, Saenger P, Horikawa R. *Once-weekly somapacitan in growth hormone deficiency: 4-year efficacy and safety results from REAL 3, a randomized controlled phase 2 trial*. Atlanta, Georgia, USA: ENDO. (2022).

25. Savendahl L, Battelino T, Rasmussen MH, Brod M, Saenger P, Horikawa R. Effective GH replacement with once-weekly somapacitan vs daily GH in children with GHD: 3-year results from REAL 3. *J Clin Endocrinol Metab* (2021) 29:29. doi: 10.1210/clinem/dgz310

26. Juul Kildemoes R, Hojby Rasmussen M, Agerso H, Overgaard RV. Optimal monitoring of weekly IGF-I levels during growth hormone therapy with once-weekly somapacitan. *J Clin Endocrinol Metab* (2021) 106(2):567–76. doi: 10.1210/clinem/dgaa775

27. Lin Z, Shu AD, Bach M, Miller BS, Rogol AD. Average IGF-1 prediction for once-weekly lonapegsomatropin in children with growth hormone deficiency. *J Endocrine Soc* (2022) 6(1):bvab168. doi: 10.1210/jendso/bvab168

28. Zelinska N, Iotova V, Skorodok J, Malievsky O, Peterkova V, Samsonova L, et al. Long-acting c-terminal peptide-modified hGH (MOD-4023): Results of a safety and dose-finding study in GHD children. *J Clin Endocrinol Metab* (2017) 102 (5):1578–87. doi: 10.1210/jc.2016-3547

29. Bidlingmaier M, Schilbach K. The use of IGF-I to monitor long-acting growth hormone therapy-timing is an art. *J Clin Endocrinol Metab* (2021) 106(5): e2367–9. doi: 10.1210/clinem/dgab016

30. Allen DB, Backeljauw P, Bidlingmaier M, Biller BM, Boguszewski M, Burman P, et al. GH safety workshop position paper: a critical appraisal of recombinant human GH therapy in children and adults. *Eur J Endocrinol* (2016) 174(2):P1–9. doi: 10.1530/EJE-15-0873

31. Christiansen JS, Backeljauw PF, Bidlingmaier M, Biller BM, Boguszewski MC, Casanueva FF, et al. Growth hormone research society perspective on the development of long-acting growth hormone preparations. *Eur J Endocrinol* (2016) 174(6):C1–8. doi: 10.1530/EJE-16-0111

32. Park P, Cohen P. The role of insulin-like growth factor I monitoring in growth hormone-treated children. *Hormone Res* (2004) 62 Suppl 1:59–65. doi: 10.1159/000080760

33. Cohen P, Rogol AD, Howard CP, Bright GM, Kappelgaard AM, Rosenfeld RG, et al. Insulin growth factor-based dosing of growth hormone therapy in children: a randomized, controlled study. *J Clin Endocrinol Metab* (2007) 92 (7):2480–6. doi: 10.1210/jc.2007-0204

34. Chatelain P, Malievskiy O, Radziuk K, Senatorova G, Abdou MO, Vlachopapadopoulou E, et al. A randomized phase 2 study of long-acting TransCon GH vs daily GH in childhood GH deficiency. *J Clin Endocrinol Metab* (2017) 102(5):1673–82. doi: 10.1210/jc.2016-3776

35. Savendahl L, Battelino T, Brod M, Hojby Rasmussen M, Horikawa R, Juul RV, et al. Once-weekly somapacitan vs daily GH in children with GH deficiency: Results from a randomized phase 2 trial. *J Clin Endocrinol Metab* (2020) 105(4):01. doi: 10.1210/clinem/dgz310

36. Raman S, Grimberg A, Waguespack SG, Miller BS, Sklar CA, Meacham LR, et al. Risk of neoplasia in pediatric patients receiving growth hormone therapy–a report from the pediatric endocrine society drug and therapeutics committee. *J Clin Endocrinol Metab* (2015) 100(6):2192–203. doi: 10.1210/jc.2015-1002

37. Allen DB, Merchant N, Miller BS, Backeljauw PF. Evolution and future of growth plate therapeutics. *Hormone Res Paediatrics* (2021) 94(9-10):319–32. doi: 10.1159/000520812

38. Hwang JS, Lee HS, Lee KH, Yoo HW, Lee DY, Suh BK, et al. Once-weekly administration of sustained-release growth hormone in Korean prepubertal children with idiopathic short stature: A randomized, controlled phase II study. *Hormone Res Paediatrics* (2018) 90(1):54–63. doi: 10.1159/000489262

39. ClinicalTrials.gov. *A research study to compare somapacitan once a week with norditropin® once a day in children who need help to grow (REAL 8)* (2022). Available at: https://clinicaltrials.gov/ct2/show/NCT05330325?term=NCT05330325&draw=2&rank=1 (Accessed 6/27/2022).

40. Djambas Khayat C. Once-weekly prophylactic dosing of recombinant factor IX improves adherence in hemophilia b. *J Blood Med* (2016) 7:275–82. doi: 10.2147/JBM.S84597

41. Jose B, Tahrani AA, Piya MK, Barnett AH. Exenatide once weekly: clinical outcomes and patient satisfaction. *Patient Prefer Adherence* (2010) 4:313–24. doi: 10.2147/ppa.s7494

42. Li A, Goodfriend C, Sokol J, Kruse-Jarres R. Patterns and predictors of emicizumab adherence in people with hemophilia. *Blood* (2019) 134 (Supplement_1):2178. doi: 10.1182/blood-2019-128083

43. Qiao Q, Ouwens MJ, Grandy S, Johnsson K, Kostev K. Adherence to GLP-1 receptor agonist therapy administered by once-daily or once-weekly injection in patients with type 2 diabetes in Germany. *Diabetes Metab Syndrome Obes Targets Ther* (2016) 9:201–5. doi: 10.2147/DMSO.S99732

44. Miller BS, Rosenfeld RG. Monitoring rhGH safety: rhGH registries, SAGhE and future needs. *Pediatr Endocrinol Rev* (2018) 16(Suppl 1):150–61. doi: 10.17458/per.vol16.2018.mr.monitoringrhghsafety

45. Geffner ME, Ibanez L, Maniatis A, La Torre D, Huang C, Darendeliler F, et al. *Pfizer registry of outcomes in growth hormone RESearch (PROGRES): a multi-country, non-interventional, prospective, cohort study of patients receiving human growth hormone treatments under routine clinical care*. Atlanta, Georgia, USA: ENDO (2022).

frontiers | Frontiers in Endocrinology

TYPE Mini Review
PUBLISHED 24 August 2022
DOI 10.3389/fendo.2022.989404

OPEN ACCESS

EDITED BY
Rodolfo A. Rey,
Centro de Investigaciones Endocrinológicas "Dr. César Bergadá"(CEDIE) (CONICET), Argentina

REVIEWED BY
George Arthur Werther,
Royal Children's Hospital, Australia
Stefano Cianfarani,
University of Rome Tor Vergata, Italy

*CORRESPONDENCE
Robert Rapaport
Robert.Rapaport@mountsinai.org

†These authors have contributed equally to this work

‡These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 08 July 2022
ACCEPTED 02 August 2022
PUBLISHED 24 August 2022

CITATION
Beliard K, Wu V, Samuels J, Lipman T and Rapaport R (2022) Identifying and addressing disparities in the evaluation and treatment of children with growth hormone deficiency.
Front. Endocrinol. 13:989404.
doi: 10.3389/fendo.2022.989404

Identifying and addressing disparities in the evaluation and treatment of children with growth hormone deficiency

Kara Beliard[1†], Vickie Wu[1†], Julie Samuels[1†], Terri H. Lipman[2‡] and Robert Rapaport[1*‡]

[1]Division of Pediatric Endocrine and Diabetes, Mount Sinai Kravis Children's Hospital, New York, NY, United States, [2]Department of Family and Community Health, University of Pennsylvania School of Nursing, Philadelphia, PA, United States

Health disparities are a significant cause of concern globally and in the United States. Disparities have been additionally highlighted throughout the ongoing COVID-19 pandemic during which populations of color have been the most affected by the disease. Social determinants of health, race, ethnicity, and gender have all contributed to disparate outcomes and disparities spanning all age groups. Multiple socio-ecological factors contribute to disparities and different strategies have been proposed. The purpose of this paper is to provide an overview of disparities in pediatric treatment and outcomes, with a focus on children with endocrine disorders.

KEYWORDS

growth hormone deficiency, pediatric short stature, gender disparities, racial disparities, healthcare disparities

Introduction

Health disparities are a significant cause of concern globally and in the United States. Disparities have been additionally highlighted throughout the ongoing COVID-19 pandemic during which populations of color have been the most affected by the disease (1, 2). Social determinants of health, race, ethnicity, and gender have all contributed to disparate outcomes and disparities spanning all age groups. Multiple socio-ecological factors contribute to disparities and different strategies have been proposed. The purpose of this paper is to provide an overview of disparities in pediatric treatment and outcomes, with a focus on children with endocrine disorders.

Health disparities are defined as differences in health outcomes that can be attributed to social, economic, or environmental disadvantages and frequently greatly affect health outcomes. For example, food insecurity in children leads to malnutrition, poor growth, weakened immune systems, and is a common cause of death globally (3). Most pediatric

health disparity research has focused on the cumulative and synergistic impact of differences in socioeconomic status (SES), race, and ethnicity on the life-course trajectory and its outcomes in adulthood.

The Center for Disease Control (CDC) defines the social determinants of health as circumstances in which individuals are born, live, work, and age that impact health outcomes (3). This encompasses four categories of interacting factors: 1) socioeconomic circumstances, 2) psychosocial factors, 3) neighborhood environment, and 4) political, economic, and cultural drivers (4, 5).

Racial and ethnic disparities exist in health care and health outcomes in the U.S. across the socioeconomic spectrum. Minority groups have been found to have a higher incidence of complications and mortality associated with oncologic diseases, chronic diseases, and infant mortality (5, 6). Most recently, the COVID-19 pandemic has significantly and disproportionately impacted minority groups in the U.S. in prevalence, intensive care unit admissions, and deaths - exacerbating pre-existing disparities (7). Given that the 2020 U.S. Census Bureau reports non-White individuals comprise 42.2% of the population, the estimated number of individuals affected is staggering (8).

Prevalent gender disparities in healthcare are another major source of high impact inequities (9). For example, despite females having an increased survival post liver transplant compared to males, females have a lower probability of receiving a liver transplant (10). Compared to men with diabetes, women with diabetes have higher rates of coronary heart disease, stroke, depression, anxiety, and mortality (11).

Health disparities in pediatric health care

Health care disparities have been extensively reported in the pediatric population. In pediatric emergency rooms children from minority groups had longer wait times and fewer analgesics prescribed in trauma cases (12). In Neonatal Intensive Care Units (NICU), compared to Black and Hispanic children, White children experienced higher rates of breastfeeding, more early intervention referrals, more kangaroo care, and had less risk of intraventricular hemorrhage, and lower rates of mortality and morbidity (13). Female infants compared to males had higher rate of post-cardiac surgery mortality (14). Even in pediatric clinical trials, representation of Black children has increased in the last decade, although still underrepresented, however disparities still exist in the enrollment of American Indian/Alaska Native, Asian, and Native Hawaiian/Pacific Islander children (15).

Health disparity in pediatric endocrinology

Social, ethnic, and gender disparities are also noted in the field of Pediatric Endocrinology. Diabetes mellitus is one of the most common pediatric chronic diseases and significant advances have been made in managing this disease (16). Recent studies have demonstrated that Black children are significantly less likely to use continuous glucose monitors and insulin pumps (17–22). Data have shown that caregivers' perception of cost and providers' perception of family competence were essential factors when deciding the level of treatment intensity in patients with type 1 diabetes mellitus (T1DM) (23). When using health insurance as a proxy for SES, White children with public insurance were 1.4 to 1.7 times more likely than Black children with commercial insurance to be prescribed an insulin pump (17). Even when both parents completed high school and college, 68% of White children received diabetes technology compared to 34% of Black children (20).

Patients who receive less intensive treatment have poorer diabetes control. Studies on rates of complications associated with T1DM in minority groups reported that Hispanic and Black children have poorer metabolic control when compared to White children (24–27). White children were found to have lower hemoglobin A1c when compared to Black children, independent of insurance type (17). When assessing chronic complications of T1DM such as diabetic retinopathy, White children were more likely to obtain annual dilated eye examination screenings than Black children (28). Compared to White and Hispanic children, Black children have more hospital admissions for diabetic ketoacidosis and more hypoglycemia episodes (18). Black children represent 46% of all pediatric diabetes mellitus population yet comprise 77% of the diabetes-related deaths. In contrast, White children represent 26% of the pediatric diabetes mellitus population and comprise only 7% of related deaths (18).

Racial and gender disparities are also seen in the evaluation of childhood short stature (SS). SS is defined as height less than two standard deviations (-2 SD) below the mean for age and sex. Growth failure is defined as growth velocity <0 SD below the mean for age and sex. SS can be a normal variant of growth as seen in familial short stature and constitutional delay; however, it can also reflect pathological states (29–31). For a child to be evaluated for endocrine causes of SS, a referral is typically initiated by the primary care provider (PCP). Retrospective data analyses have reported a predominance of White males being referred for concerns of growth (22, 32). Caregivers' attitudes and level of concern play an essential role in deciding when to refer a child to a subspecialty clinic and the degree of concern is not uniform amongst different families. When parents were questioned about the impact of SS on adult men

and women, they reported that short men suffer in self-esteem and personal success; in contrast, short women were not believed to face these problems (33). Several studies have found that Black families had a higher threshold to consider SS an issue, believing that height is a minor problem when more important issues exist (34, 35). Explicit and implicit providers' bias has been extensively reported and even the most well-meaning providers may have subconscious biases, known as implicit biases (7, 36). It is therefore crucial for health care providers to identify and address structural racism in health care team that may be perpetuating poor health outcomes in minority groups.

In the evaluation of children with SS associated with growth hormone deficiency (GHD), data from several growth hormone (GH) surveillance programs have highlighted gender, racial and ethnic disparities in diagnosis and treatment (Table 1). August et al. reported the demographics of children followed in the post-marketing surveillance of Somatrem; the population consisted of 87.8% White, 6% Black, and 1% Asian (40). Males comprised 71.6% of their population and females comprised 28.4%. They noted that Black children referred for SS were shorter and had lower peak GH levels during the growth hormone stimulation test (GHST) than their White counterparts. At diagnosis, females with idiopathic GHD were significantly shorter than males (-3.9 ± 1.3 SD versus -3.3 ± 1.4 SD, respectively). The KIGS worldwide registry (Pfizer International Growth Database) gathered data from 1987-2012 and included over 80,000 children with SS; of those evaluated and treated for GHD, 70% were White, 14.4% were East Asian, 1.1% were Black, and 2.4% were Hispanic, with a higher frequency of males in total (70.1% males versus 29.9% females) (41). Studies have reported that male White children and children from higher annual family income and parental education were more likely to be evaluated for SS, and be diagnosed and treated for GHD (33, 43). In one study, 91% of the families presenting for evaluation of their child's SS were White and 5% were Black, yet the regional population is 72% White and 25% Black; in addition, 49% of the presenting families had annual incomes ≥ $50,000 however only 23% of families in the county had this level of income (33).

Compared to females, males are more likely to be screened for GHD by PCPs (44). White male children are referred more often for SS evaluation when compared to minority groups and females (37). As part of the evaluation for SS, Pediatric Endocrinologist may perform a GHST. This test is performed after an overnight fast and involves administering provocative agents (clonidine, arginine, glucagon, insulin) and obtaining serial GH concentrations. Disparities are seen with GHST, with more White males proceeding with the test than minority groups and females (37). These studies also highlight the high proportion of females not being assessed for SS; this is distressing as SS can be the only physical examination finding in patients with Turner Syndrome (38, 39, 42, 44, 45). Grimberg et al. reported a higher rate of pathological/organic causes of SS in females in their evaluation, even when excluding Turner Syndrome as a cause (32).

Proposed interventions

Over the years, multiple authors have highlighted these health disparities and proposed different interventions to reduce and eventually eliminate disparities in healthcare. The underrepresentation of minority children and females being evaluated and treated for short stature due to GHD is striking and demands attention.

We propose the following interventions to address the aforementioned issues, focusing on pediatric GHD Figure 1.

- EDUCATE FAMILIES REGARDING THE IMPORTANCE OF HEALTH MAINTENANCE VISITS FOR CHILDREN
  - o The recommended schedule for health maintenance visits is at the first week of life, 1 month, 2 months, 4 months, 6 months, 9 months, 12 months, 15 months, 18 months, 24 months, 30 months, 3 years, and annually thereafter.
  - o Families should be educated about the importance of scheduling and attending the health maintenance visits.
- DEMAND KNOWLEDGE OF GROWTH PARAMETERS LONGITUDINALLY
  - o At every visit with the PCP, families should receive information about their child's growth parameters – both the absolute measurements and the percentiles for age and sex.
- WHEN GROWTH PATTERNS DEVIATE, STRESS THE IMPORTANCE OF EVALUATION
  - o When growth patterns deviate, evaluation is crucial to distinguish between normal variants of growth such as constitutional delay of growth, conditions unrelated to hormonal causes such as poor nutrition or celiac disease, and endocrinopathies such as GHD.
- EDUCATE COMMUNITY PROVIDERS REGARDING APPROPRIATE EVALUATION AND REFERRAL OF CHILDREN WITH CONCERNS FOR GHD
  - o The decision to refer a child for an evaluation for endocrine causes of SS should be standardized. The American Academy of Pediatrics (AAP) recommends monitoring growth parameters (height and weight) at each health maintenance visit as an effective general health and well-being assessment method.
  - o Correct height measurement techniques must be implemented to prevent growth failure from being unrecognized or misdiagnosed (46). All health care team members responsible for obtaining height measurements should receive training on proper technique and equipment.

TABLE 1 Gender and Racial disparities in the diagnosis and treatment of Growth Hormone Deficiency.

Table 1A

Paper	M:F % referral	M:F height z-score at time of referral
Hawkes et al. (37)	61/39	
Kamoun et al. (38)	65/35	-1.8/-2.0
Grimberg et al. (32)	65/35	-1.9/-2.4
Tanaka et al. (39)	61.3/38.7	-2.47/-2.52

Table 1B

Paper	W:B % referrals
Hawkes et al. (37)	79/11
Kamoun et al. (38)	90/10

Table 1C

Paper	M:F %, underwent GHST to diagnose IGHD	M:F %, diagnosed with IGHD	M:F %, received GH treatment for all GH indications	M:F %, received GH treatment for IGHD	M:F %, received GH treatment for ISS	M:F baseline height z-score, received GH treatment for IGHD
Grimberg et al. (34)			63/37			
August et al. (40)			70.5/29.5	71.6/28.4		-3.3/-3.9
Ranke et al. (41)			67/33	70/30	71/29	-2.84/-3.22
Kamoun et al. (38)	70/30			70/30		-2.2/-2.5
Hughes et al. (42)			64/36	62/38	66/34	-2.092/-1.6
Tanaka et al. (39)	45.7/49.8	61.4/38.6				

Table 1D

Paper	W:B %, underwent GHST to diagnose IGHD	W:B %, received GH treatment for all GH indications	W:B %, received GH treatment for IGHD	W:B %, received GH treatment for ISS	W:B, baseline height z-score, received GH treatment for IGHD	W:B, Maximal GH peak in ng/mL on GHST, received GH treatment for IGHD
Grimberg et al. (34)		83/4	84/3	85/3	-2.6/-3.0	6.0/4.9
August et al. (40)		87.8/6.0			-3.4/-4.1	3.5/2.8
Hawkes et al. (37)	84/9		83/10		-2.3/-2.5	7.2/4.7

A- Percentage of females (F) and males (M) referred to Pediatric Endocrinology for short stature assessment with their corresponding initial height z-scores. B- Percentage of White (W) and Black (B) children referred to Pediatric Endocrinology for short stature assessment. C- Percentage of females (F) and males (M) assessed and treated for Growth Hormone Deficiency with their corresponding initial height z-scores. D- Percentage of White (W) and Black (B) children assessed and treated for Growth Hormone Deficiency with their corresponding initial height z-scores and Growth Hormone peak during the Growth Hormone Stimulation Test (GHST).
- IGHD, Idiopathic Growth Hormone Deficiency.
- GH, Growth Hormone.
- GHST, Growth Hormone Stimulation Test.
- ISS, Idiopathic Short Stature.

- o When children exhibit abnormal growth patterns, PCPs must perform a proper diagnostic evaluation that may include referral to a Pediatric Endocrinologist.
- o The PCP's decision to pursue evaluation of SS should be irrespective of a child's gender or race.

- EDUCATE PARENTS AND CAREGIVERS REGARDING GHD, ITS COMPLICATIONS, AND TREATMENT OPTIONS
- o Parental education should focus on complications associated with childhood GHD, including the impact

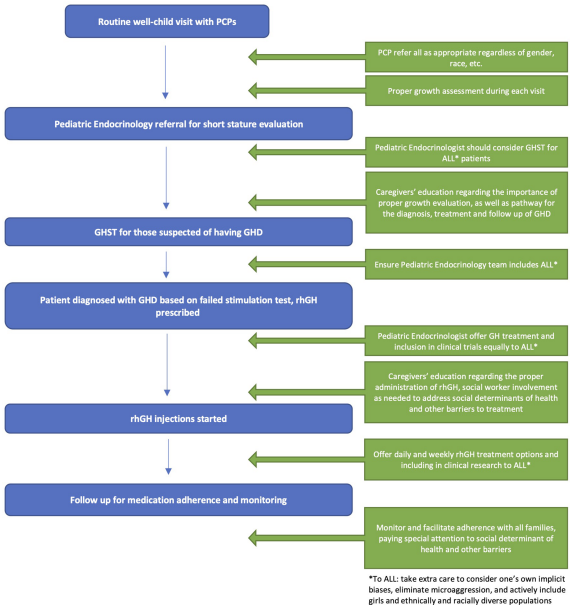


FIGURE 1
Proposed Interventions.

on bone health, lipid profile, psychosocial state, and well-being.

o The indications for recommending GHST should be clearly explained to families and all questions should be addressed to limit concerns.

o Parents may have misconceptions regarding the safety of recombinant human GH. The medication has been historically associated with Creutzfeldt-Jakob disease linked to contaminated human GH obtained from the cadaveric human pituitary gland. Cases of Creutzfeldt-Jakob disease associated with GH treatment are no longer a concern since the transition to recombinant human GH (rhGH) in 1985 (47).

o Families may be hesitant to start the medication for fear of daily injections. However, weekly rhGH is available and FDA approved to be used in the pediatric population.

• EDUCATE PEDIATRIC ENDOCRINOLOGISTS WITH LATEST GHD RESEARCH AND TREATMENT OPTIONS

o Pediatric Endocrinologists should be knowledgeable about the latest GHD research and treatment options.

o Pediatric Endocrinologists should also be knowledgeable in how to administer rhGH to appropriately address patients' and families' questions or troubleshoot issues with administration.

• ADDRESSING THE DISPARITIES OBSERVED IN GHST WHEN CLINICALLY INDICATED

o GHST should be offered to all children in whom GHD is suspected, regardless of race, ethnicity, or gender.

o The decision to undergo GHST should not be influenced by parental perceptions of height outcomes in males versus females. Providers should stress the indications for GHST and the importance of treatment of GHD not just for height attainment.

o Provider bias, if present, should be recognized and should not interfere with the recommendation of GHST if clinically warranted.

o Pediatric Endocrinologists should strive to identify their own potential biases and barriers to offering and providing equal care to males and females, regardless of race, ethnicity, and SES.

• EVALUATION WITH MRI

o After the clinical and biochemical diagnosis of GHD is made, obtaining magnetic resonance imaging (MRI) is recommended to evaluate the hypothalamic pituitary region. It can identify pituitary abnormalities such as anterior pituitary hypoplasia, posterior pituitary ectopia, and pituitary stalk agenesis. MRI can also exclude the presence of a pituitary tumor.

o The role of MRI should be clearly explained to the family and recommended to all patients if indicated.

- GH TREATMENT INITIATION
  - o GH therapy should be offered equally to all patients who meet the diagnostic criteria for GHD.
- SUPPORT DURING GH TREATMENT
  - o Promoting inclusivity amongst families with children with GHD is encouraged. Connecting families with a new diagnosis of GHD with families who are actively receiving or who have completed treatment with rhGH to establish/promote more community support may ease the caregivers' concerns.
  - o If there are financial or social barriers to initiating GH treatment, the Pediatric Endocrinology office should have effective intervention options in place, such as social workers available to identify resources to help mitigate these concerns.
- MONITORING OUTCOMES DURING AND AFTER GH TREATMENT
  - o Follow-up visits in the office are typically every three months for close monitoring of height, side effects, and dose adjustments. Efforts should be made to support patients and caregivers for them to be able to attend these visits in the form of appointment availability and flexibility.
  - o When GH treatment is discontinued, patients and families should continue to follow up at the Pediatric Endocrinology office.
- PROMOTE DIVERSITY OF THE HEALTH CARE TEAM
  - o Efforts must be made to increase diversity in the health care team – in ethnicity, race, and gender.

Discussion

Health care disparities have been a subject of discussion for decades however we have yet to find the best way to address this ongoing concern. Culturally competent, equitable care that is sensitive to patients' needs should be the priority. The patients' health literacy and cultural beliefs should be considered when discussing these matters with the families (7). Well-meaning providers may have implicit biases that may impact their decision when referring children for SS evaluation, proceeding with GHST, and ensuring appropriate treatment and follow-up for different patient populations. Providers' unintentional more positive attitudes towards White patients and negative attitudes towards ethnically and racially diverse groups can impact patient care (48, 49). Many have postulated that racial diversity in the medical field is an essential step in addressing racial disparities. Healthcare providers need to examine their own practices to ensure elimination of unconscious or overt biases that can perpetuate microaggression in the patient-provider relationship. It is imperative that providers address structural racism and its role in perpetuating health disparities (22).

Health care disparities have significantly and disproportionally impacted minority populations. This is seen in various areas of health care, but the conclusion remains unchanged, with underrepresented groups having worse outcomes. Different solutions have been postulated – this includes educating caregivers, improving the social determinants of health of patients, educating, and diversifying healthcare providers, and addressing and alleviating implicit and explicit bias in healthcare providers. In the field of pediatric endocrinology, we propose steps to advance equity in the evaluation, diagnosis, and treatment of children for GHD. A multidisciplinary approach is needed to minimize implicit or explicit bias, to encourage collaboration between members participating in patient care, and to support families through the treatment of GHD.

Author contributions

KB and VW wrote the manuscript. JS contributed to the editing as well as the research and addition of reference material. TL and RR helped develop the initial outline and significantly contributed to the content and editing of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Parpia AS, Martinez I, El-Sayed AM, Wells CR, Myers L, Duncan J, et al. Racial disparities in COVID-19 mortality across Michigan, United States. *EClinicalMedicine* (2021) 33:100761. doi: 10.1016/j.eclinm.2021.100761

2. Agrawal H, Das N, Nathani S, Saha S, Saini S, Kakar SS, et al. An assessment on impact of COVID-19 infection in a gender specific manner. *Stem Cell Rev Rep* (2021) 17(1):94–112. doi: 10.1007/s12015-020-10048-z

3. A hunger for action. *Lancet* (2022) 399(10340):1991. doi: 10.1016/S0140-6736(22)00975-8

4. Walker RJ, Strom Williams J, Egede LE. Influence of race, ethnicity and social determinants of health on diabetes outcomes. *Am J Med Sci* (2016) 351(4):366–73. doi: 10.1016/j.amjms.2016.01.008

5. Wheeler SM, Bryant AS. Racial and ethnic disparities in health and health care. *Obstet Gynecol Clin North Am* (2017) 44(1):1–11. doi: 10.1016/j.ogc.2016.10.001

6. Maina IW, Belton TD, Ginzberg S, Singh A, Johnson TJ. A decade of studying implicit racial/ethnic bias in healthcare providers using the implicit association test. *Soc Sci Med* (2018) 199:219–29. doi: 10.1016/j.socscimed.2017.05.009

7. Magesh S, John D, Li WT, Li Y, Mattingly-App A, Jain S, et al. Disparities in COVID-19 outcomes by race, ethnicity, and socioeconomic status: A systematic-review and meta-analysis. *JAMA Netw Open* (2021) 4(11):e2134147. doi: 10.1001/jamanetworkopen.2021.34147

8. Jensen E, Jones N, Rabe M, Pratt B, Medina L, Orozco K, et al. *The chance that two people chosen At random are of different race or ethnicity groups has increased since 2010*. United States Census Bureau New York (2022). Available at: https://www.census.gov/library/stories/2021/08/2020-united-states-population-more-racially-ethnically-diverse-than-2010.html.

9. Darden M, Parker G, Anderson E, Buell JF. Persistent sex disparity in liver transplantation rates. *Surgery* (2021) 169(3):694–9. doi: 10.1016/j.surg.2020.06.028

10. Burra P, De Martin E, Gitto S, Villa E. Influence of age and gender before and after liver transplantation. *Liver Transpl* (2013) 19(2):122–34. doi: 10.1002/lt.23574

11. Kautzky-Willer A, Harreiter J, Pacini G. Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. *Endocr Rev* (2016) 37(3):278–316. doi: 10.1210/er.2015-1137

12. LaPlant MB, Hess DJ. A review of racial/ethnic disparities in pediatric trauma care, treatment, and outcomes. *J Trauma Acute Care Surg* (2019) 86(3):540–50. doi: 10.1097/TA.0000000000002160

13. Sigurdson K, Mitchell B, Liu J, Morton C, Gould JB, Lee HC, et al. Racial/Ethnic disparities in neonatal intensive care: A systematic review. *Pediatrics* (2019) 144(2):e20183114. doi: 10.1542/peds.2018-3114

14. Klitzner TS, Lee M, Rodriguez S, Chang RK. Sex-related disparity in surgical mortality among pediatric patients. *Congenit Heart Dis* (2006) 1(3):77–88. doi: 10.1111/j.1747-0803.2006.00013.x

15. Rees CA, Stewart AM, Mehta S, Avakame E, Jackson J, McKay J, et al. Reporting of participant race and ethnicity in published US pediatric clinical trials from 2011 to 2020. *JAMA Pediatr* (2022) 176(5):e220142. doi: 10.1001/jamapediatrics.2022.0142

16. Sperling MA, Laffel LM. Current management of glycemia in children with type 1 diabetes mellitus. *N Engl J Med* (2022) 386(12):1155–64. doi: 10.1056/NEJMcp211217

17. Lipman TH, Hawkes CP. Racial and socioeconomic disparities in pediatric type 1 diabetes: Time for a paradigm shift in approach. *Diabetes Care* (2021) 44(1):14–6. doi: 10.2337/dci20-0048

18. Lado JJ, Lipman TH. Racial and ethnic disparities in the incidence, treatment, and outcomes of youth with type 1 diabetes. *Endocrinol Metab Clin North Am* (2016) 45(2):453–61. doi: 10.1016/j.ecl.2016.01.002

19. Lai CW, Lipman TH, Willi SM, Hawkes CP. Racial and ethnic disparities in rates of continuous glucose monitor initiation and continued use in children with type 1 diabetes. *Diabetes Care* (2021) 44(1):255–7. doi: 10.2337/dc20-1663

20. Willi SM, Miller KM, DiMeglio LA, Klingensmith GJ, Simmons JH, Tamborlane WV, et al. Racial-ethnic disparities in management and outcomes among children with type 1 diabetes. *Pediatrics* (2015) 135(3):424–34. doi: 10.1542/peds.2014-1774

21. Lipman TH, Smith JA, Patil O, Willi SM, Hawkes CP. Racial disparities in treatment and outcomes of children with type 1 diabetes. *Pediatr Diabetes* (2021) 22(2):241–8. doi: 10.1111/pedi.13139

22. Hawkes CP, Lipman TH. Racial disparities in pediatric type 1 diabetes: Yet another consequence of structural racism. *Pediatrics* (2021) 148(2):e2021050333. doi: 10.1542/peds.2021-050333

23. Valenzuela JM, La Greca AM, Hsin O, Taylor C, Delamater AM. Prescribed regimen intensity in diverse youth with type 1 diabetes: Role of family and provider perceptions. *Pediatr Diabetes* (2011) 12(8):696–703. doi: 10.1111/j.1399-5448.2011.00766.x

24. Gallegos-Macias AR, Macias SR, Kaufman E, Skipper B, Kalishman N. Relationship between glycemic control, ethnicity and socioeconomic status in Hispanic and white non-Hispanic youths with type 1 diabetes mellitus. *Pediatr Diabetes* (2003) 4(1):19–23. doi: 10.1034/j.1399-5448.2003.00020.x

25. Delamater AM, Shaw KH, Applegate EB, Pratt IA, Eidson M, Lancelotta GX, et al. Risk for metabolic control problems in minority youth with diabetes. *Diabetes Care* (1999) 22(5):700–5. doi: 10.2337/diacare.22.5.700

26. Delamater AM, Albrecht DR, Postellon DC, Gutai JP. Racial differences in metabolic control of children and adolescents with type I diabetes mellitus. *Diabetes Care* (1991) 14(1):20–5. doi: 10.2337/diacare.14.1.20

27. Auslander WF, Thompson S, Dreitzer D, White NH, Santiago JV. Disparity in glycemic control and adherence between African-American and Caucasian youths with diabetes. family and community contexts. *Diabetes Care* (1997) 20(10):1569–75. doi: 10.2337/diacare.20.10.1569

28. Dumser SM, Ratcliffe SJ, Langdon DR, Murphy KM, Lipman TH. Racial disparities in screening for diabetic retinopathy in youth with type 1 diabetes. *Diabetes Res Clin Pract* (2013) 101(1):e3–5. doi: 10.1016/j.diabres.2013.03.009

29. Graber E, Rapaport R. Growth and growth disorders in children and adolescents. *Pediatr Ann* (2012) 41(4):e1–9. doi: 10.3928/00904481-20120307-07

30. Teran E, Chesner J, Rapaport R. Growth and growth hormone: An overview. *Growth Horm IGF Res* (2016) 28:3–5. doi: 10.1016/j.ghir.2016.02.004

31. Rapaport R, Wit JM, Savage MO. Growth failure: 'idiopathic' only after a detailed diagnostic evaluation. *Endocr Connect* (2021) 10(3):R125–38. doi: 10.1530/EC-20-0585

32. Grimberg A, Kutikov JK, Cucchiara AJ. Sex differences in patients referred for evaluation of poor growth. *J Pediatr* (2005) 146(2):212–6. doi: 10.1016/j.jpeds.2004.09.009

33. Finkelstein BS, Singh J, Silvers JB, Marrero U, Neuhauser D, Cuttler L. Patient attitudes and preferences regarding treatment: GH therapy for childhood short stature. *Horm Res* (1999) 51 Suppl 1:67–72. doi: 10.1159/000053138

34. Grimberg A, Lindberg A, Wajnrajch M, Cucchiara AJ, Camacho-Hübner C. Racial/Ethnic disparities in US pediatric growth hormone treatment. *Horm Res Paediatr* (2018) 90(2):102–8. doi: 10.1159/000491793

35. Lipman TH, McCurry IJ. Children with short stature and growth failure: Heightism, gender and racial disparities. *Pediatr Endocrinol Rev* (2017) 14(Suppl 2):472–7. doi: 10.17458/per.vol14.2017.lm.childrenshortstature

36. Chapman EN, Kaatz A, Carnes M. Physicians and implicit bias: how doctors may unwittingly perpetuate health care disparities. *J Gen Intern Med* (2013) 28(11):1504–10. doi: 10.1007/s11606-013-2441-1

37. Hawkes CP, Gunturi H, Dauber A, Hirschhorn JN, Grimberg A. Racial and ethnic disparities in the investigation and treatment of growth hormone deficiency. *J Pediatr* (2021) 236:238–45. doi: 10.1016/j.jpeds.2021.04.034

38. Kamoun C, Hawkes CP, Gunturi H, Dauber A, Hirschhorn JN, Grimberg A. Growth hormone stimulation testing patterns contribute to sex differences in pediatric growth hormone treatment. *Horm Res Paediatr* (2021) 94(9-10):353–63. doi: 10.1159/000520250

39. Tanaka T, Soneda S, Sato N, Kishi K, Noda M, Ogasawara A, et al. The Boy: Girl ratio of children diagnosed with growth hormone deficiency-induced short stature is associated with the Boy:Girl ratio of children visiting short stature clinics. *Horm Res Paediatr* (2021) 94(5-6):211–8. doi: 10.1159/000518995

40. August GP, Lippe BM, Blethen SL, Rosenfeld RG, Seelig SA, Johanson AJ, et al. Growth hormone treatment in the United States: Demographic and diagnostic features of 2331 children. *J Pediatr* (1990) 116(6):899–903. doi: 10.1016/s0022-3476(05)80647-x

41. Ranke MB, Lindberg A, Tanaka T, Camacho-Hübner C, Dunger DB, Geffner ME. Baseline characteristics and gender differences in prepubertal children treated with growth hormone in Europe, USA, and Japan: 25 years' KIGS® experience (1987-2012) and review. *Horm Res Paediatr* (2017) 87(1):30–41. doi: 10.1159/000452887

42. Hughes IP, Choong CS, Cotterill A, Harris M, Davies PS. Gender bias in children receiving growth hormone treatment. *J Clin Endocrinol Metab* (2010) 95(3):1191–8. doi: 10.1210/jc.2009-1563

43. Hitt T, Ginsburg KR, Cousounis P, Lipman TH, Cucchiara AJ, Stallings VA, et al. Concerns and expectations of parents seeking subspecialist care for their child's short stature. *Horm Res Paediatr* (2019) 92(5):311–8. doi: 10.1159/000506739

44. Grimberg A, Feemster KA, Pati S, Ramos M, Grundmeier R, Cucchiara AJ, et al. Medically underserved girls receive less evaluation for short stature. *Pediatrics* (2011) 127(4):696–702. doi: 10.1542/peds.2010-1563

45. Grimberg A, Huerta-Saenz L, Grundmeier R, Ramos MJ, Pati S, Cucchiara AJ, et al. Gender bias in U.S. pediatric growth hormone treatment. *Sci Rep* (2015) 5:11099. doi: 10.1038/srep11099

46. Lipman TH, Hench KD, Benyi T, Delaune J, Gilluly KA, Johnson L, et al. A multicentre randomised controlled trial of an intervention to improve the accuracy of linear growth measurement. *Arch Dis Child* (2004) 89(4):342–6. doi: 10.1136/adc.2003.030072

47. Ranke MB. Short and long-term effects of growth hormone in children and adolescents with GH deficiency. *Front Endocrinol (Lausanne)* (2021) 12:720419. doi: 10.3389/fendo.2021.720419

48. Hall WJ, Chapman MV, Lee KM, Merino YM, Thomas TW, Payne BK, et al. Implicit Racial/Ethnic bias among health care professionals and its influence on health care outcomes: A systematic review. *Am J Public Health* (2015) 105(12):e60–76. doi: 10.2105/AJPH.2015.302903

49. Mulchan SS, Wakefield EO, Santos M. What COVID-19 teaches us about implicit bias in pediatric health care. *J Pediatr Psychol* (2021) 46(2):138–43. doi: 10.1093/jpepsy/jsaa131

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership