Differential Diagnosis of the Short IGF-I-Deficient Child with Apparently Normal Growth Hormone Secretion

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Abstract
The current differential diagnosis for a short child with low insulin-like growth factor I (IGF-I) and a normal growth hormone (GH) peak in a GH stimulation test (GHST), after exclusion of acquired causes, includes the following disorders: (1) a decreased spontaneous GH secretion in contrast to a normal stimulated GH peak (“GH neurosecretory dysfunction,” GHND) and (2) genetic conditions with a normal GH sensitivity (e.g., pathogenic variants of \textit{GH1} or \textit{GHSR}) and (3) GH insensitivity (GHI). We present a critical appraisal of the concept of GHND and the role of 12- or 24-h GH profiles in the selection of children for GH treatment. The mean 24-h GH concentration in healthy children overlaps with that in those with GH deficiency, indicating that the previously proposed cutoff limit (3.0–3.2 μg/L) is too high. The main advantage of performing a GH profile is that it prevents about 20% of false-positive test results of the GHST, while it also detects a low spontaneous GH secretion in children who would be considered GH sufficient based on a stimulation test. However, due to a considerable burden for patients and the health budget, GH profiles are only used in few centres. Regarding genetic causes, there is good evidence of the existence of Kowarski syndrome (due to \textit{GH1} variants) but less on the role of \textit{GHSR} variants. Several genetic causes of (partial) GHI are known (\textit{GHR}, \textit{STAT5B}, \textit{STAT3}, \textit{IGF1}, \textit{IGFALS} defects, and Noonan and 3M syndromes), some responding positively to GH therapy.

Introduction
In the diagnostic approach of the short and/or slowly growing child, serum insulin-like growth factor I (IGF-I) is considered one of the essential components of the laboratory screening procedure, as a first indication for growth hormone (GH) deficiency (GHD) or insensitivity (GHI) [1–5]. If serum IGF-I is low (or in the lower half of the reference range) for age, sex, and pubertal stage, the next step is usually to assess the endogenous GH reserve by a GH stimulation test (GHST). The results of 2 GHSTs,
performed separately or combined on the same day [6], serve as a proxy indicator of GH secretion. If the diagnosis of GHD is established, magnetic resonance imaging of the hypothalamic/pituitary region is indicated to search for the anatomical cause [7].

Unfortunately, there are important drawbacks with the use of both diagnostic tools (serum IGF-I and GHST). The assessment of the circulating IGF-I concentration is hampered by different assays and standards, and there is scarcity of reference data, particularly for pubertal stage [2, 4, 8, 9]. Also, GHSTs suffer from serious disadvantages, as already highlighted more than 25 years ago [10], and we wish to highlight 6 examples.

First, different pharmacological agents generate different mean GH peak sizes, with poor correlations among tests [11–16]. For example, the peak GH in response to pharmacological stimulation with clonidine is significantly higher than the response to arginine, insulin [17], and glucagon [16]. Second, there is no published guidance on differentiating the cutoff of the GH peak in a GHST between prepubertal and pubertal children, while GH peaks are considerably higher in puberty due to increased sex hormone (mainly oestrogen) concentrations [18, 19]. Third, there is no consensus on the use and timing of sex steroid priming in prepubertal young teenagers [7], despite the recommendation in the recent US guideline [6]. Fourth, a high BMI (probably mainly due to high fat mass, leading to increased rate of degradation, reviewed in [20]) has a negative effect on the GH peak [21], but it is unknown how to adjust for this effect. Fifth, the cutoff for the GH peak is arbitrary, and the change in conversion factor from the GH bioassay (mU/L) to mass units (μg/L) by increasing purity of the GH preparations has further complicated the situation. This is illustrated by the change of the cutoff for a “normal” GH peak over time, from 15 mU/L (then equivalent to 7.5 μg/L) via 20 mU/L (10 μg/L) to 30 mU/L (equivalent to 10 μg/L when a more pure laboratory standard [1 mg = 3 IU] had been introduced) and back to approximately 20 mU/L (6.7–7 μg/L) [7, 22]. Finally, the reproducibility of a GHST is low [14], probably caused partially by the effect of a variable interval between the pharmacological agent and the foregoing spontaneous GH pulse (the refractory interval is estimated at 3 h [23, 24]). All these uncertainties have led to a large variation in clinical practice around the world with regard to GHSTs [25, 26].

If in a short child with a low serum IGF-I, a “classical” GHD is excluded by a normal stimulated GH peak, one should first try to assess whether the sensitivity to GH is normal or decreased. In the excellent review by Storr et al. [27], GHI was defined as “impairment of all or some of the mechanisms of physiological GH action.” According to this definition, all disorders of the GH-IGF axis except GHD belong to this diagnostic category, irrespective of the serum IGF-I concentration, also including some syndromes with dysmorphic features (e.g., Noonan and three M [3M] syndromes), IGF2 defects, and GH neutralizing antibodies in patients with a GHI gene deletion. We prefer to rather call this “decreased responsiveness” to GH and prefer to restrict the term GHI to disorders with a low serum IGF-1 concentration in spite of a normal GH secretion, so only including disorders of the GH receptor and post-receptor signalling and its main target hormones (IGF-I and acid-labile subunit [ALS]). We thus exclude disorders of the GH-IGF axis with a normal or increased serum IGF-I caused by genetic defects of IGF1R, IGF2, or PAPPA2 and genetic disorders affecting post-receptor signalling of the IGF-1 receptor [28], which still fall under the umbrella of GH unresponsiveness.

The logical diagnostic step to assess GH sensitivity is to measure the change in circulating IGF-I after a certain period of GH administration. Initially, this was used to identify children who would be expected to respond positively to GH injections [29–31]. Later, when recombinant human IGF-1 (rhIGF) became available, the test was named “IGF-1 generation test” (IGFGT) and mainly used to estimate the likelihood of Laron syndrome [32]. That regimen consisted of a series of 4 daily GH injections of a single dosage (33 μg/kg - day). Later, more complex regimens of the IGFGT were investigated, consisting of several periods of escalating GH dosages [33, 34]. At present, multiple regimens are being used and the sensitivity and specificity to detect molecularly proven cases of GHI are reportedly high, although “the ability of the IGFGT to detect less severe GHI is doubtful” [35].

Over the past 15 years, the IGFGT has been used in the Netherlands in a standardized format for children with severe short stature (height less than −2.5 standard deviation score [SDS]), persistent low IGF-1 (less than −2 SDS) and a normal stimulated GH peak (>10 μg/L), using a schedule of 1, 2 or 3 escalating GH doses (0.7, 1.4 and 2.8 mg/m² - day) for 1 week each, with minimal intervals of 4 weeks. If the child’s serum IGF-I SDS response is <1 SD, the response is assessed on a higher GH dose [26, 36]. This scheme results in 4 categories of GH sensitivity: normal (sufficient response to lowest GH dose), moderate insensitivity (sufficient response to intermediate dose), modest insensitivity (sufficient response to highest dose), and (near-) complete insensitivity (no response to any dose).
This minireview aims at discussing the differential diagnosis for a short child with low IGF-I and a normal GH peak in at least one GHST, in whom acquired causes of decreased serum IGF-I (e.g., malnutrition, hypothyroidism, psychosocial dwarfism, and anorexia nervosa) have been ruled out. In Critical Appraisal of the Diagnostic Value of GH Profiles and of the Validity of the Concept of GH Neurosecretory Dysfunction, we present a critical appraisal of the role of 12- or 24-h GH profiles in the diagnosis of short stature and of the concept of growth hormone neurosecretory dysfunction (GHND). In Genetic Conditions Associated with Normal GH Sensitivity, several genetic conditions associated with a normal GH sensitivity are discussed, in particular pathogenic GH1 and GHSR variants. Causes of GHI are reviewed in Conditions with Decreased GH Sensitivity. In Hypothetical Causes of Short Stature Associated with a Low Serum IGF-I and Normal Stimulated GH Peak, we speculate on some hypothetical causes.

Critical Appraisal of the Diagnostic Value of GH Profiles and of the Validity of the Concept of GH Neurosecretory Dysfunction

In the early 1970s, the use of a small portable continuous blood withdrawal system [37] enabled performing spontaneous 24-h GH profiles, either by the continuous withdrawal technique [37] or frequent sampling [38]. For a comparison of these methods, the reader is referred to [20]. The 24-h GH profiles show a circadian rhythm (related to the sleep-wake cycle) and an ultradian rhythm regulated by the central nervous system and are also influenced by exercise, stress, and the daily feeding cycle [39]. A pilot study in short children suggested that some short children with normal GHST results might have a decreased spontaneous GH secretion [40]. Furthermore, a disturbed secretory pattern with decreased GH secretory spikes, both in frequency and amplitude, were observed as a result of cranial irradiation, first in rhesus monkeys [41] and later in children recovered from acute lymphatic leukaemia or brain tumours [42–47]. A later study suggested that a significant number of patients developed hypothalamic radiation-induced damage to the GHRH-secreting neurons, and secondary to this a decreased responsiveness to GHRH in the pituitary gland [48].

Theoretically, a spontaneous 24-h GH profile would appear a better test to assess GH secretion. However, the taskforce of the Drug and Therapeutics, and Ethics Committees of the Pediatric Endocrine Society felt that “any potential benefit of overnight GH sampling did not warrant the burden to patients” [6]. Still, in a 2002 European audit, routine assessment of spontaneous GH secretion was reported by 40 respondents (17%) [25], and according to a recent audit in 8 European countries, 12-h nocturnal GH profiles are still used in Germany and the UK [26]. In Sweden, clinicians currently have 2 options: a spontaneous 12-h GH night profile or a GHST (arginine-insulin tolerance test, AITT) preceded by a 3-h GH profile (to avoid false-positive test results) [24, 49, 50].

Characteristics of 24-h GH Profiles in Healthy Children

Looking back at the 3 decades in which most scientific work was done on GH profiles in children (1980–2010), the different interpretations of the diagnostic value of GH profiles seem to be essentially based on different perceptions of the reference range of GH secretion in healthy children, the influence of limited sample sizes, the reproducibility, and the chosen outcome measure, including the role of the GH secretory pattern.

Determinants, Reference Range, and Sample Sizes

Essentially, spontaneous GH profiles can be used to assess the GH secretion pattern or the GH secretion rate. The major determinant of spontaneous GH secretion rate in healthy children is pubertal stage: GH secretion increases with advancing puberty, with a peak at Tanner stage 4 [17, 20, 51–55]. Further, mean night-time GH levels correlate inversely with BMI in both sexes [53, 55], in line with an inverse correlation between GH secretion rate per kg body mass and weight for height SDS in pubertal children [56]. Spontaneous GH secretion is positively correlated with height SDS [56] and height velocity [57].

Two groups of clinical investigators (headed by Bercu and Zadik, respectively) concluded that healthy controls and short children with normal height velocity had a 24-h integrated concentration of GH (IC-GH) above 3.0 or 3.2 μg/L, respectively [17, 58, 59]. However, this conclusion was based on very few control subjects. Spiliotis et al. [58] coined the term GHND, defined by the following criteria: height less than first percentile; height velocity <4 cm/year; bone age ≥2 years behind chronological age; normal findings from GHST (peak >10 μg/L); low somatomedin-C (IGF-I) level; and abnormal 24-h GH secretory pattern. However, the number of prepubertal control children with short stature was only 9. In the follow-up study [59], it was an unreported fraction of 31 controls (short and
normal stature) with a mean age of 13.2 years (range 5.0–17.9 years) and a Tanner stage range of 1–5. In the study by Zadik et al. [17], 95% of IC-GH values in all 119 children were above 3.2 μg/L, but no information was provided on the percentage in the 36 prepubertal children.

Two other groups concluded that 24-h GH secretion in prepubertal controls is quite variable and overlaps with observations in children with GHD: the group headed by Albertsson-Wikland in Gothenburg (Sweden) [52] and Rose et al. at NIH (Bethesda, USA) [53, 60]. In 62 prepubertal children, mean 24-h GH concentration was close to 2 μg/L [53]. Besides these 4 groups, 24-h GH profiles were performed in several other centres such as London (Hindmarsh/Brook) and Tübingen (Bierich/Ranke).

Reproducibility

In 40 poorly growing children in whom a GH profile was performed twice within 4 weeks, the first and second IC-GH were highly correlated, but at the individual level, there were quite large differences [15]. In another study on 24-h GH profiles in 9 children with a mean time interval of 1.5 years, the intra-individual reproducibility was rather poor: the difference in secretion ranged from −31% to +37%, with a mean intra-individual coefficient of variance of 12%. The changes in mean peak amplitudes between the repeated profiles showed a considerable inter-individual variation between −54% and +38% (mean coefficient of variance 15%) [61]. Similar results were obtained in other longitudinal studies [62, 63]. Albertsson-Wikland and Rosberg [20] concluded “that the reproducibility of repeated 24-h profiles is nearly as low as the reproducibility of the GH response to repeated pharmacological tests for an individual child; if there are any differences, they are less pronounced for 24-h integrated concentrations than for the GH response to pharmacological tests” [15, 62, 64].

Choice of Outcome Measure

A 24-h GH profile can be based on integrated (continuous) versus discrete sampling (usually at 20-min intervals), and software programs generate a number of output variables [20, 52, 65]. For example, the Pulsar analyses with setting for 24-h GH curves generate data on overall mean; the maximal and minimal value; the mean of the calculated baseline; the number of peaks; the mean interpeak interval; the mean peak height, length, amplitude, and peak area; and the area under the curve above the zero level as well as above the calculated baseline [52]. The usual outcome measure is an indicator of the average GH concentration at all time points, such as the IC-GH, mean GH concentration, or area under the curve. However, in the construction of a prediction model for the growth response to recombinant human GH (rhGH) treatment, the highest GH peak (usually occurring at night) showed a slightly better correlation with the growth response than the area under the curve above baseline, estimated as roughly equivalent to the mean GH level [49, 66].

Critical Appraisal of the Concept of GH Neurosecretory Dysfunction

Based on a pilot study on the IC-GH in short children [40] and the study on children with acute lymphatic leukaemia who underwent preventive irradiation [42], Spiliotis et al. [58] investigated 32 short children and 13 normal-stature controls. Of the short children, 16 were classified as GHD (GH peak <10 μg/L in a GHST, mean 24-h GH concentration <0.6–3.3 μg/L), 9 as controls (normal GH peaks after stimulation, mean 24-h GH concentration 3.1–12.2 μg/L), and 7 as GHND (normal GH peak in stimulation test, mean GH concentration ≤3 μg/L). When one takes a closer look at the 7 patients with GHND, all were in Tanner stage 1, while this was only expected for 3 children aged 7.4, 11.6, and 12.5 years. The remaining 4 males (aged 14.8, 15.0, 15.0, and 15.5 years) had an extremely delayed puberty and bone age. It is noteworthy that the highest nocturnal GH peak was ≥10 μg/L in 6 out of 16 in the GHD group and in all members of the GHND group (10–22 μg/L). Treatment of the GHND patients with pituitary hGH (3/x/week) doubled height velocity, similar to the “GHD” patients.

In a follow-up study [59] on 73 patients (21 GHD, 21 GHND, 18 short controls, and 13 normal-stature controls), GHND was now formally defined as a mean serum 24-h GH concentration below 3.0 μg/L, a normal response (>10 μg/L) to provocative testing, a low plasma IGF-I, and clinical features consistent with GHD. Again, in all children labelled GHND, the highest nocturnal GH concentration was >10 μg/L, close to the peak GH in the stimulation test [59]. Similar results were reported in 18 children with GHND [66]. GHND was also found in 3 out of 5 children with an empty sella [67].

In the meantime, Dr. Zadik had published similar data on IC-GH of 90 short children (19 GHD) compared with IC-GH from 46 children of normal stature. Mean IC-GH in children with GHD, short children with a normal GH peak in a GHST, and normal-stature controls was 1.6 ± 0.6, 3.8 ± 2.3, and 6.6 ± 1.9 μg/L, respectively. Forty-five percent of children with normal stimulated GH responses had an IC-GH within the range of values for the group.
with GHD [68]. The incidence of GHND (IC-GH <3.2 μg/L) among children with a height SDS less than −2.5 without obvious underlying causes was estimated at 45% [69]. The authors also showed that differences in IC-GH between normally growing and poorly growing children are due to a lower amplitude of peaks during the daytime hours [70] and that rhGH administration did not suppress endogenous GH secretion in patients with GHND [71]. This was later confirmed by Lundberg et al. [72], who showed spontaneous GH peaks for 4 h following sc GH injections. The growth response of boys with GHND after 4 years of GH treatment was similar to that of children with classical GHD [73], and GH treatment had a positive effect on adult height [74]. Interestingly, at retesting after 3–4 years, 3 children with GHND showed a subnormal GH response to stimulation, suggesting that in some patients, a regulatory defect in neurosecretion is noted first, while at a later stage, the response of the pituitary to stimulation is lost [75].

We believe that there are several reasons to challenge the claims of these 2 groups on the existence of the hypothetical GHND. First, the distribution of the mean GH concentration of healthy children is much wider than the authors’ estimations, probably associated with the limited number of prepubertal controls. The 3.0–3.2 μg/L cutoff for IC-GH is too high, according to observations in larger groups of children [52, 53]. Second, the authors probably overestimated the reproducibility. Third, the authors have not given proper attention to an alternative outcome marker that may be at least as important, that is, the highest spontaneous GH peak [49], which was >10 μg/L in all cases [58, 59]. In addition, the growth response to hGH treatment in children labelled as GHND does not support the concept of a separate GHND condition either. Bercu and colleagues showed that the response to pituitary hGH administration (0.15–0.30 mg/kg · week, 3–7 injections/week) was similar in short children regardless of provoked and/or endogenous GH secretory dynamics [76–78].

Does this mean that “GHND” does not exist? We believe there may be 2 examples of its existence in special pathological conditions. First, the robust data on children who received cranial irradiation indicate a decreased spontaneous GH secretion and disturbed GH secretion pattern, as mentioned above [42–47]. Second, there is a clinical syndrome in which spontaneous GH secretion and serum IGF-I are usually decreased, while GHST results are often normal: Prader-Willi syndrome [79, 80]. For example, in 23 non-obese children, the mean stimulated GH peak was approximately 6 μg/L (implying that approximately 40% would have a peak of >7 μg/L), 4 times lower than healthy controls [81], and mean 24-h GH concentration was 0.7 μg/L [82], suggesting that a GHST is not an appropriate test in such children. In a recent Dutch study, mean serum IGF-I in childhood was −1.7 SDS, suggesting that IGF-I was less than −2 SDS in about 40% of cases. Interestingly, GH secretion appears to increase by age in this syndrome: both serum IGF-I and IGFBP-3 normalized in GH-treated young adults, and none of the patients met the criteria for adult GHD [83].

Besides these 2 examples, the use of 2 different tools for assessing GH secretion (a GH profile and a GHST) automatically leads to groups, which are labelled deficient with one tool and not with the other. This will be further discussed in the following sections.

Critical Appraisal of the Diagnostic Value of Spontaneous GH Secretion

In contrast with the 2 groups of clinical scientists that assumed healthy children have an IC-GH above 3.0–3.2 μg/L (see Critical Appraisal of the Concept of GH Neurosecretory Dysfunction), 2 other groups emphasized the wider range of GH secretion and the considerable overlap with children with GHD [52, 53]. However, the conclusions of the 2 latter groups differed. Rose et al. [84] concluded that “standard GH stimulation tests, despite their limitations, remain the best definitive test of GH secretion,” which ended the NIH programme. In contrast, the group of Albertsson-Wikland and her successors considered a 12-h GH profile superior to a GHST. As mentioned earlier, in Sweden such profile or a GHST preceded by a 3-h GH profile to control for refractoriness are currently used in Sweden (personal communication Drs. Albertsson-Wikland and Kriström).

The current approach of this Swedish group is based on a series of studies between the early 1980s and 2007, in which spontaneous GH secretion was used as a potential predictor in the growth “prediction model.” The Göttingen prediction models were designed to predict the growth response in the first 2 years of GH treatment (expressed as the change in height SDS) in short children irrespective of GH secretion, so including GHD and idio-pathic short stature (ISS). In addition to data included into the basic model data (auxological data from the year before the start of GH treatment and parental heights), the other 4 models included either growth data from the first 2 years of life, serum IGF-I, GH secretion estimated during a provocation test (AITT) or a spontaneous GH secretion profile [49]. While the GH peak during the AITT or IGF-I SDS were predictive (though at an inter-
mediate magnitude, similar to the predictive value of early growth), the maximum GH peak over 24 h (GH_{max24h}) was the most informative variable, closely followed by the area under the curve above baseline. Adding early growth variables and GH_{max24h} to the basic model resulted in the model with the best accuracy (narrower prediction interval), considerably better than if the GH peak during the AITT provocation tests or IGF-I SDS was added [49]. A further refinement of the prediction model, including data from children born prematurely and/or small for gestational age (SGA), was published in 2007 [85]. The original prediction model [49] was used to test the hypothesis whether individualized GH doses, based on variation in GH responsiveness estimated by the prediction model, reduced variability in growth response around a preset height target compared with a standardized weight-based dose, in 153 short prepubertal children who were tested with a 24-h GH profile as well as an AITT. If the highest GH peak from the 24-h profile was used instead of the GH peak in the AITT, the diagnosis was switched from isolated GHD to ISS in 30% of cases [50]. Individualized GH doses during catch-up growth indeed reduced the proportion of unexpectedly good and poor responders around a predefined individual growth target, while mean growth responses were equal [50].

Several studies have assessed to which extent a shorter interval than 24 h would generate similarly useful information. The highest GH peak obtained during a sampling period limited to 12 h at night resulted in an acceptable prediction level for 97% of the children and was still a better predictor of the growth response than the GH peak during an AITT or IGF-I levels when used in the prediction models [86]. Shortening the time interval to 6 h resulted in a considerable loss of accuracy [86, 87]. Technical aspects of the analysis of 24-h GH profiles (secretion rate and pattern) were described in detail [56, 65] as well as the influence of standard preparations, GH isoforms, assay characteristics, GH-binding protein (GHBP) [22] and 24-h profiles in short children born SGA [88].

In the discussion about whether one should use the GH peak in a GHST or an indicator of spontaneous GH secretion (or both), there is a tension between theoretical and pragmatic arguments. In theory, a 12- or 24-h GH profile should be a better indicator of spontaneous GH secretion than the GH peak in a GHST, considering all its drawbacks [10]. However, the burden to the patient and the financial and logistical burden to the health and hospital system are considered larger. We assume that these are the main reasons that presently GH profiles are used only in few centres.

We believe that for centres where GH profiles are not done, it is still helpful to be informed about observations in centres performing 24-h GH profile as well as a GHST. First, it is informative to see the scatterplot of the highest GH peak in the 24-h GH profile (GH_{max24h}) versus the GH peak observed in the AITT, as shown in Figure 1, kindly provided by Drs. Berit Kristrom and Albertsson-Wikland et al. [49]. The figure was first published in the PhD thesis of Dr. Kristrom, Umeå University Medical Dissertations, ISBN 91–7191–611–3, and kindly provided by Dr. Kristrom. GH, growth hormone; GH_{max24h}, highest GH peak in a 24-h GH profile; GH_{maxAITT}, GH peak in an arginine-insulin tolerance test.
test only, and 19% pathological results on the stimulation test only. At a cutoff of 10 μg/L, these percentages were 34, 37, 11, and 18%, respectively [24]. These results show that a potential advantage of this strategy is that the 12- or 24-h GH profile can reduce the number of false-positive tests of GHSTs by approximately 20%. The authors provided evidence that this discrepancy is frequently caused by the refractory interval of 3 h [23]. At the same time, in 7% of short children, a low nocturnal GH peak is found in contrast to a normal GH peak in a GHST [24], who may respond positively to GH treatment [24]. Interestingly, a similar percentage was found in Tübingen (Germany), where 9% of non-acquired cases with GHD were labelled GHND [89]. While it is tempting to assume that these cases indeed represent GHND, one should still bear in mind the suboptimal reproducibility of GH profiles as well as GHSTs [61, 63]. A reassuring observation is that the stimulated GH peak can be reliably replaced by the 12-h GH peak with similar accuracy in the KIGS prediction model [90].

Conclusion

If for logistical or financial reasons, no GH profile for a short child with a low serum IGF-I and normal GHST can be performed, the clinician can choose among 3 options. First, one can decide to prescribe rhGH as a therapeutic trial, if national regulations permit, for example, in Sweden [91, 92] and the USA. Second, one can conclude that the child is not GH deficient and abstain from any treatment. A third option was taken by the paediatric endocrine community in the Netherlands, where an IGFGT treatment. A third option was taken by the paediatric endocrine community in the Netherlands, where an IGFGT is performed in such cases; if the IG-F-I response is sufficient on any dose, a 1-year trial with rhGH therapy is allowed by the National Committee, which may be continued if the growth response is appropriate [26].

Genetic Conditions Associated with Normal GH Sensitivity

Kowarski Syndrome (Bioinactive GH Protein, Caused by a Pathogenic GH1 Variant)

While in the majority of IGF-I-deficient children with a normal result of the GHST or spontaneous GH secretion, the cause may be associated with the inaccuracy of both tests, there can also be a genetic origin. The 2 main candidate genes for such presentation include GH1, encoding the pituitary GH protein, and GHSR, encoding the GH secretagogue receptor (ghrelin receptor).

Most pathogenic variants of GH1 cause a form of congenital GHD, either with an autosomal recessive inheritance pattern (isolated GHD types 1A [MIM #262400] or 1B [MIM #612781]) or an autosomal dominant inheritance (isolated GHD type 2, MIM #173100) [93–95]. Patients with isolated GHD type 1A have severe growth retardation, which usually becomes apparent in the first 6 months of life and is caused by homozygous or compound heterozygous deletions, insertions, frameshift, or nonsense variants in GH1. In patients with isolated GHD type 1B, serum GH levels are low but detectable and the phenotype is more heterogeneous. In most cases, homozygous or compound heterozygous splice site, frameshift, nonsense, or missense variants in GH1 or GHRHR (encoding the GH-releasing hormone receptor) are found. In isolated GHD type 2, with autosomal dominant inheritance, GH secretion is very low but usually still detectable, and in most cases, it is associated with heterozygous intronic deletions, missense, splice site, or splice enhancer variants in GH1 [94, 96, 97].

Kowarski syndrome (MIM #262650) was named after the American paediatric endocrinologist who studied 2 unrelated boys with growth retardation and delayed bone ages, presenting with a normal immunoreactive GH peak after stimulation, but low levels of serum IGF-I [98]. Their growth and serum IGF-I responded positively to GH administration. At that time, further genetic assessment could not be performed. In the following years, several patients with similar clinical features were reported [29, 99–101].

Proof of the existence of a clinical syndrome caused by a bioinactive GH protein was provided in 1996–1997 [102–104] (Table 1). The first case with clinical and laboratory features of severe GHD, but with an increased GH peak in an insulin tolerance test (38 μg/L), was reported to carry a p.(Arg77Cys) variant (c.307C>T, p.(Arg-103Cys) according to the HGVS nomenclature) [102, 103, 105]. The mutant GH did not stimulate tyrosine phosphorylation in IM-9 cells and also inhibited the ability of wild-type GH (wt-GH) to stimulate tyrosine phosphorylation, thus having a dominant negative action. The affinity of the mutant GH for GHBP was significantly higher than that of wt-GH. Interestingly and unexplained, in the proband’s father carrying the same variant, isoelectric focussing revealed that the father’s serum contained a single GH peak corresponding to wt-GH.

The second case with a milder phenotype was reported to carry a heterozygous p.(Asp112Gly) variant (HGVS c.335A>G, p.(Asp112Gly)) [104]. The locus of this variant was found within site 2 of the GH molecule in binding sites 1 and 2 of the GH molecule.
with the GH receptor and GHBP, and the expressed recombinant mutant GH tended to form a 1:1 instead of the 1:2 GH–GHBP complex normally produced by wt-GH. The authors concluded that this variant molecule is bioinactive by preventing dimerisation of the GH receptor. After these seminal papers, several more cases have been reported, and the clinical and laboratory data of all presently known GH1 mutations associated with bioinactive GH syndrome are shown in Table 1 [102–114].

In an interesting study on children with short stature, reduced height velocity, and bone age delay [106] and a cohort of children with severe GHD, there was one child with a normal GH peak in a GHST and his short mother who carried a heterozygous GH1 variant reported as p.(Ser71Phe) (HGVS c.290C>T, p.(Ser97Phe)). Although segregation of the variant was not confirmed and although no data were reported on serum IGF-I and the growth response to GH, in vitro studies showed that the

| GH1 (NM_000515.4) Variant (HGVS) a | Reported as | Age, years | Height SDS at diagnosis | GHmax, µg/L | IGF-I SDS | GH therapy response b | Comments | Reference |
|-----------------------------------|-------------|------------|-------------------------|------------|----------|-----------------------|----------|-----------|
| c.236G>C p.(Cys79Ser)             |             | 9          | −3.6                    | 44.7       | −3.4     | +/good                | Absence of disulphide bridge; reduced affinity for GHR | [109] |
| c.253C>T p.(Pro59Ser)             |             | 9.9        | −4.6                    | 17         | −2.8     |                       | Lower affinity for binding to GHR and JAK2/STAT5 signalling | [114] |
|                                   |             | 8.0        | −5.5                    | 11         | −3.0     |                       | | |
| c.254C>T p.(Pro85Leu)             |             | 7.7        | −2.5                    | 6.9, 5.6   | −2.4     | +/good                | Decreased GH secretion and lower affinity for binding to GHR and JAK2/STAT5 signalling | [113] |
|                                   |             | 13.6       | −2.0                    | 4.7, 4.8   |           | +/good                | | |
| Adult                             |             |            |                         |            |          |                       | | |
| c.290C>T p.(Ser71Phe)             |             | 13.5       | −3.8                    | 13.6       |          |                       | No data on segregation. Reduces ability to activate JAK2/STAT | [107] |
| c.307C>T p.(Arg103Cys)            |             | 5.6        | −6.1                    | 38; 15; 35 | −2.1     | +/poor                | Inhibition of binding of wt-GH, mutant was not expressed in father | [103, 104, 106] |
|                                   |             | 37         | −0.2                    | 23.7       | −1       |                       | | |
| c.307C>T p.(Arg103Cys)            |             | 6–20       | −2.5 to −1.9             | 28         | −3.1 to −2.2 |                       | Less induction of GHR/GHBP | [110] |
|                                   |             | 32         | −1.4                    | 32         | −2.1     |                       | | |
|                                   |             | 64         | −1.7                    | 26         | −2.3     |                       | | |
| c.413A>G p.(Asp138Gly)            |             | 3          | −3.6                    | 26; 41; 51 | −2.5     | +/good                | Mutant prevents dimerisation | [105] |
| c.611G>A p.(Arg204His)            |             | 5–10       | −6.0 to −7.2             | 3.9        | −2.3     | +/moderate (+3.2 SD)  | Affects GH secretion, binding and signalling | [112] |
| c.615C>G p.(Ile205Met)            |             | 6.9        | −2.7                    | 10.4       | −1.2     |                       | No cosegregation, normal activation of JAK-STAT. Activation of ERK reduced to half | [108] |
| c.615C>G p.(Ile205Met)            |             | 4.7        | −2.0                    | 5.9        |          | +/good                | No functional studies; segregation with phenotype | [115] |
| c.626G>A p.(Arg209His)            |             | 2.1–9.8    | −4.0 to −2.6             | 5.5–10.9   | −4.5 to −2.5 |                       | Two siblings carrying same variant have isolated GHD | [117] |
| n = 4                             |             |            |                         |            |          |                       | | |

Adapted from [95, 96]. Compared with our previous publication [96], 2 variants, p.(Ser134Arg) and p.(Thr201Ala), were omitted, because these were GH deficient. GH, growth hormone; IGF-I, insulin-like growth factor I; SDS, standard deviation score; GHBP, GH-binding protein; GHD, growth hormone deficiency. a The transcript that was used for HGVS nomenclature was NM_000515.4, and the protein reference sequence was NP_000506.2. The c. denotes nucleotide position on cDNA with the A of the translation start site (ATG) of the cDNA numbered +1. b A positive growth response is indicated as “+.” c The GH peak was reported as 27.2 mU/L, at that time equivalent to 13.6 μg/L.
IGF-I Deficiency in Short Stature

A recent paper in a large extended family showed that a heterozygous GH1 variant known to be associated with type II GHD (c.626G>A, p.(Arg209His), previously reported as p.(Arg183His)), can in some individuals also lead to a presentation of short stature with a normal GHST and good growth response to rhGH, suggestive for Kowarski syndrome [116].

In conclusion, there is no doubt that some heterozygous GH1 variants encode variant GH molecules that are bioinactive and can have a dominant negative effect. In a few cases, GH secretion is also affected, at first sight suspected for isolated GHD type 2, and in other cases, it may be combined with partial GHI. The syndrome is characterized by clinical features and low serum IGF-I and IGFBP-3 concentrations compatible with GHD, in contrast with a normal or even increased serum GH response to a GHST, and the growth response to rhGH treatment in terms of growth and serum IGF-I is generally appropriate.

Ghrelin Insensitivity (GHSR Defects)

In 1999, ghrelin was isolated as the endogenous ligand for the receptor GHSR1A (encoded by GHSR) and for its ability to stimulate GH secretion [117]. In addition to a direct stimulatory effect on the pituitary gland, ghrelin was shown to amplify GH secretion by modulation of the activity of GHRH neurons [118]. Consistent with the physiological actions of acyl-ghrelin on energy homeostasis and GH secretion, animals with a disruption in GHSR display a leaner and shorter phenotype and have reduced IGF-I levels [119], supporting a role of the GHSR in body growth [118]. Two GHSR isoforms have been identified [120, 121]; the primary GHSR1A product contains 7 transmembrane domains, whereas GHSR1B is an inactive form with 5 transmembrane domains [122].

Although “isolated partial GHD” due to a GHSR variant is a registered syndrome (MIM #615925), only few cases have been reported, the clinical and laboratory phenotype is remarkably diverse, and cosegregation of the genotype with short stature is little convincing [123, 124]. A summary of reported cases is shown in Table 2. GHSR variants have also been associated with obesity [125].

The first case was found in a genetic analysis in 43 children with “short normal stature,” probably equivalent to ISS. The novel heterozygous genetic variant c.837C>A, p.(Phe279Leu), led to the exchange of a highly conserved amino acid in the sixth transmembrane domain of GHSR [125], which had been previously described to exert de-
increased specific binding properties for a GHSR agonist [126]. Further support for the pathogenicity of the variant was offered by the observation that the variant was inherited via the mother with short stature [125].

The second variant, c.611C>A, p.(Ala204Glu), was reported in 2 unrelated families from Morocco [127]. In family 1, the proband (with a homozygous mutation) was very short (height −3.7 SDS) and had a normal GHST result. Of the 5 relatives heterozygous for the variant, the parents and 2 siblings were short, but 1 sibling had a normal stature. In family 2, the heterozygous proband had isolated GHD (height –3.2 SDS), but only the parent carrying the GHSR variant was short, while height of the 2 siblings (also carriers) was well within the reference range, one even +1.0 SDS. The authors suggested that this variant is dominant with a penetrance of 66%. In vitro, the variant resulted in decreased cell surface expression of the receptor and selectively impaired the constitutive activity of the GHSR, while preserving its ability to respond to ghrelin [127]. The high basal activity of GHSR has shown to be of physiological importance in regulating both GH secretion and food intake, as demonstrated with in vivo experiments (reviewed in Wang and Tao [128]). The functional effect of the variant was recently confirmed in a GHSR-Ala203Glu mutant mouse model, which also showed decreased body weight, body length, and femur length at 1 year of age [129].

In a later publication by the French group [130], a boy with partial isolated GHD (height −2.7 SDS) was found to be compound heterozygous for 2 GHSR variants. The 2 heterozygous carriers of the p.(Trp2*) nonsense variant (mother and 1 sibling, height −1.3 and −1.7 SDS, respectively) tended to be shorter than the carrier of the p.(Arg237Trp) missense variant (father, height −0.6 SDS).

### Table 2. Selected characteristics of patients with GHSR (NM_198407.2) variants, sorted according to year of publication

| cDNA change | Protein change | gnomAD (v2.1.1) MAF | Zygosity | N | Height SDS (mean, range) | GH peak in GHST, µg/L | Diagnosis | Country | Reference |
|-------------|----------------|---------------------|----------|---|-------------------------|-----------------------|-----------|---------|-----------|
| c.837C>A    | p.(Phe279Leu)  | 0.0053%             | Het      | 1 | Less than −2.0          |                       | ISS       | Germany | [126]     |
| c.611C>A    | p.(Ala204Glu)  | 0.0028%             | Hom      | 1 | −3.7                    | 9.7–16                | ISS       | Morocco | [128]     |
|             |                 |                     | Het      | 6 | −2.3 (−3.7 to −1.1)     | 3–4.7 in GHD          | 1 GHD, 4 ISS, 1 normal |
|             |                 |                     | Het      | 1 | −3.2                    | 1–1.7 in GHD          | GHD       |
|             |                 |                     | Het      | 3 | −1.1 (−2.0 to 1.0)      | 1 ISS, 2 normal       |
| c.6G>A      | p.(Trp2*)       | NR                  | Comp het | 1 | −2.7                    | 6–7                   | GHD       | France  | [131]     |
| c.709A>T    | p.(Arg237Trp)   | 0.020%              |          |   |                         |                       |           |         |           |
| c.107_109del| p.(Gln36del)    | 0.0058%             | Het      | 3 | −3.3 (−3.4 to −3.1)     |                       | ISS?      | Japan   | [123]     |
| c.323C>T    | p.(Pro108Leu)   | 0.00080%            | Het      | 1 | −2.8                    |                       | ISS?      |
| c.517T>C    | p.(Cys173Arg)   | NR                  | Het      | 2 | −2.4 (−2.8 to −1.9)     |                       | ISS?      |
| c.737A>C    | p.(Asp246Ala)   | NR                  | Het      | 2 | −3.1 (−3.3 to −3.0)     |                       | ISS? One wt sibling equally short |
| c.251G>T    | p.(Ser84Ile)    | NR                  | Het      | 1 | −2.4 → −0.7             | 10.3                  | ISS/CDGP  | Brazil  | [132]     |
| c.545T>C    | p.(Val182Ala)   | NR                  | Het      | 1 | −2.3 → −1.4             | 7.9                   | ISS/CDGP  |
| c.611C>A    | p.(Ala204Glu)   | 0.0028%             | Het      | 2 | −2.6; −1.7              | 4.6                   | 1 GHD, 1 normal |
| c.611C>A    | p.(Ala204Glu)   | 0.0028%             | Het      | 2 | −2.1; −1.4              | 7.5                   | 1 GHD, 1 normal |
| c.526G>A    | p.(Gly176Arg)   | NR                  | Het      | 2 | −3.2; NR                | 7.2                   | 1 ISS, 1 GHD | Czech Rep | [134]     |
| c.545T>C    | p.(Val182Ala)   | NR                  | Het      | 1 | −2.6 → −2.5             |                       | CDGP      | Brazil  | [133]     |

CDGP, constitutional delay of growth and puberty; Comp het, compound heterozygous; gnomAD, Genome Aggregation Database; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; Het, heterozygous; Hom, homozygous; ISS, idiopathic short stature; MAF, minor allele frequency; NR, not reported; SDS, standard deviation score; Rep, Republic; wt, wild type; →, longitudinal data on height SDS.
and the sibling carrying wt-GHSR (height −0.2 SDS). In vitro experiments showed that the p.(Arg237Trp) variant would result in a partial loss of constitutive activity of the receptor, whereas both its ability to respond to ghrelin and its cell surface expression were preserved.

A Japanese group performed mutational screening of GHSR in 127 unrelated Japanese patients diagnosed with either isolated GHD (n = 14) or ISS (n = 113), and 188 control subjects were analysed for the presence of these mutations [122]. For 4 variants, the clinical features and functional studies (loss of constitutive activity) made it likely that the GHSR variants may cause short stature. The heterozygous p.(Gln36del) variant was detected in 3 patients as well as 1 control and the 3 other heterozygous variants, p.(Pro108Leu), p.(Cys173Arg), and p.(Asp246Ala), were only found in a single patient each. However, data on cosegregation were limited, and in several cases not confirmatory. For the first patient with the p.(Gln36del) variant, no genetic data on the normal-stature parents were available, the parent of the second patient carrying this variant had a completely normal height (0.0 SDS), and clinical or genetic data were not available for the third patient. For the p.(Pro108Leu) variant, both parents had normal stature and their DNA was not tested. The parent and sibling carrying the variant encountered in the third patient p.(Cys173Arg) had a height of −1.9 SDS and −0.5 SDS, respectively. The proband and his mother carrying the p.(Asp246Ala) variant had a similar height SDS (−3.3 and −3.0 SDS, respectively), but the sibling carrying the wt genotype was equally short (−3.1 SDS) [122].

In a Brazilian study, the GHSR coding region was directly sequenced in 96 independent patients with ISS (31 of them with constitutional delay of growth and puberty [CDGP]), 150 adults, and 197 children with normal stature [131]. Two short girls with CDGP who reached a normal adult height were found to carry a pathogenic variant, showing a decrease in basal receptor activity, in part explained by a reduction in cell surface expression. The p.(Ser84Ile) variant was not inherited via the mother, and no genetic analysis could be performed in the father. Notably, the 2 siblings who had normal stature and puberty were also heterozygous for the same variant. The father and sister of the girl carrying the p.(Val182Ala) variant were also carriers, and their growth and puberty patterns were compatible with CDGP. Her sister was treated with rhGH for GHD (maximum GH peak at stimulation test of 1.8 μg/L) [131]. In a recent paper [132], an unrelated proband carrying this same variant was reported, with a height of −2.5 SDS at a relatively late pubertal onset of 13.5 years, with a bone age delay of 3 years [131].

In a cohort of 46 Moroccan index cases with isolated GHD, the same GHSR variant, as previously reported, p.(Ala204Glu) [127], was detected in 2 children from 2 families, but in each family, this variant was also present in 1 sibling with normal height [114]. A child with severe GHD carried a novel variant, p.(Ala358Thr), inherited from his father with a height of −2.2 SDS. Though the alanine residue at codon 358 is conserved among mammalian species, the p.(Ala358Thr) variant was reported as a rare polymorphism [131] and is now listed as single-nucleotide polymorphism (SNP, rs150344113) with a frequency of 0.6 and 1.7% in American multi-ethnic (dbSNP) and African American (Exome Variant Server) cohorts, respectively. Finally, in a study on genetic testing of children with familial short stature, a heterozygous GHSR variant, p.(Gly176Arg), was found in an SGA born child with borderline GHD and limb shortening, as well as in his younger brother born appropriate for gestational age diagnosed with GHD [133].

Besides these case reports, it is important to note that in another cohort of short children, no pathogenic GHSR variants were found [134]. As applies to most scientific information, one can expect that there is a publication bias, because negative findings are not likely to be published. In our Laboratory for Diagnostic Genome Analysis, we detected 8 GHSR variants in 9 unrelated children out of 1,698 patients referred for genetic testing by next generation sequencing for short stature and/or skeletal dysplasia (0.5%). Three variants were previously reported as pathogenic (2x c.611C>A p.(Ala204Glu); c.709A>T p.(Arg237Trp); and c.837C>A p.(Phe279Leu)) [125, 127, 130, 135]. The other 5 variants included 1 likely pathogenic truncating variant and 4 missense variants of uncertain significance (Drs. Losekoot and Van Duyvenvoorde, personal communication).

An indirect indication that GHSR variants may be associated with height is the significant association of GHSR polymorphisms with height in genome-wide association studies [136–138]. However, in another study, common variation in GHSR was not associated with body size [139]. In a study aimed at identifying genetic polymorphisms, which could serve as predictive markers of response to rhGH therapy, no such marker in GHSR was found [140]. In contrast, body length and weight of goats and pigs were significantly higher in animals carrying a GHSR polymorphism [141, 142].

In theory, a diminished function of the ghrelin receptor may occur not only as a result of a pathogenic GHSR variant but also because of a genetic abnormality of a regulator of GHSR expression. A recent paper reported that
mice with a reduced Reck expression or with induced Reck deficiency from 10 days after birth showed decreases in body size and plasma levels of IGF-I. Reck is a tumour suppressor gene encoding reversion-inducing cyto-
eine-rich protein with Kazal motifs (Reck), a membrane-
anchored protease regulator expressed in multiple tissues in mouse embryos. In postnatal Reck−/− mice, immuno-
reactivity of GH was greatly reduced, while GHSR and GHRH receptor immunoreactivity was decreased, al-
though their mRNAs were increased [143].

In conclusion, arguments in favour of the hypothesis that GHSR variants are associated with short stature and CDGP are the various case reports, GWAS studies, functional data on decreased constitutive action of GHSR, and the knock-out mice studies. Arguments against include the variability of clinical phenotypes (GHD, ISS, or CDGP) and circulating IGF-I concentrations, incomplete segregation of the variations with the phenotype (normal stature in some carriers and short stature in parents or siblings with a wt genotype), and potential publication bias, in line with our previous considerations [123, 124].

**Other Suggested Mechanisms of a Diminished Action of GH**

There are a few other potential medical conditions for which the evidence on diminished GH action is weak. These will be discussed in the following paragraphs.

**Abnormal Composition of Secreted and Circulating GH Isoproteins**

Human GH isolated and secreted from the pituitary gland is not a single protein but rather a mixture of vari-
ants (isoproteins) differing in amino acid sequence, post-
translational modified forms, and fragments [144]. The major component (22 kDa, GH molecule with a molecu-
lar weight of 22 kDa [22K-GH]) is a single-chain polypep-
tide containing 191 amino acids and 2 intra-chain disul-
phide bridges, synthesized and stored in granules of spe-
cific acidophilic cells of the anterior pituitary [145].

According to an analysis of the approximate mean distri-
bution of pituitary GH isoforms in human blood 15–30 min after a secretory pulse, 45% consists of 22K-GH, half of which is bound to high-affinity GHBP [144]. The sec-
ond main GH isoform (20K-GH), derived from GHI by alter-
ative mRNA splicing, has a structure analogous to 22K-GH, except for the deletion of internal residues 32–71. It has 176 amino acids and a molecular mass of approxi-
mately 20 kDa [146]. Five percent of the secretory pulse consists of GH molecule with a molecular weight of 20 kDa (20K-GH) [144]. Acidic GH (desamido-, acylated, and glycosylated GH) occupies 5% of the secretory pulse. Other components of the secretory pulse include 3 class-
es of dimeric GH (22K 20%, 20K 5%, and acidic GH di-
mers 2%) and 3 classes of oligomeric GH (22K 10%, 20K 2%, and acidic GH dimers 2%) [144]. A third isoform, arising from skipping of exon 3 and lacking amino acid residues 32–71, was proposed as an additional GH variant (17.5K-GH) [147] but is not expressed in significant amounts under normal conditions [144].

In initial studies, various isoforms appeared to show differences in bioactivity. For example, 20K-GH was re-
ported to lack insulin-like effects and have diminished diabetogenic activity [148]. However, subsequent studies yielded conflicting information, probably in part due to species differences. In humans, the somatogenic activity of 20K-GH appears qualitatively similar and quantita-
tively equivalent to that of 22K-GH [144]. However, the bioactivity of naturally occurring GH oligomers com-
pared to monomeric 22K-GH ranges from moderately re-
duced to full bioactivity [144].

Regarding immunoreactivity, the heterogeneity of GH is the main cause of the disparities of GH results obtained among assays and laboratories, particularly with the modern specific monoclonal assays [149]. Furthermore, the plasma half-life of endogenous 20K-GH and of mo-
nomeric, dimeric, or oligomeric GH is longer than that of 22K-GH [150]. The picture is complicated further in the circulation, where GH binds to 2 GHBP, each with different affinities for the GH isoforms.

There are 2 papers suggesting that an abnormal com-
position of GH isoproteins may cause short stature. In the first case report [151], a 14-year-old boy showed a growth pattern consistent with GHD, normal GH peaks to GHSTs, and an excellent growth response to GH admin-
istration. Plasma somatomedin C level (the previous name for IGF-I) was interpreted as normal (1.7 U/mL, reference 0.4–4.5 U/mL for age). The ratio of radiorecep-
tor-assayable to radioimmunoassayable GH was de-
creased, as well as the biological activity. When analysed by column chromatography, most of the immunoreactive GH migrated as approximately 85 kDa (“big-big”) and 45 kDa (“big”) species, and these GH polymers constituted 60–90% of all immunoreactive material [151]. At the time, the normal quantity of tetramers and dimers in plasma was estimated at 14–39% [152], in later studies slightly higher (41%) [144]. Furthermore, in the patient, almost all polymers were resistant to conversion by urea. The authors concluded that the patient’s short stature was due to an abnormal structure of his endogenous GH mol-
ecule. Our present interpretation is that the normal so-

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DOI: 10.1159/000516407
matomedin-C (IGF-I) level is a rather strong argument against this hypothesis. Genetic studies have not been reported for this patient.

The initial data on differences in bioactivity, binding properties, and metabolic clearance of the various GH isoproteins, as well as reports that different GH analogues and fragments may interact as weak agonists or antagonists of the GHR depending on the relative affinities of binding sites 1 and 2 to the GHR [153], were the reason for a Swedish group to test the hypothesis that short stature may be due to an abnormal distribution of GH isoproteins [154]. Serum non-22-kDa GH levels, expressed as a percentage of the total GH concentration, were determined by the 22-kDa GH exclusion assay. The median proportion of non-22-kDa GH isoforms was only slightly increased in children born SGA and girls with Turner syndrome but not in the group of children with ISS, compared with 23 normal-stature children (8.1%). In the SGA group, the proportion of non-22-kDa GH isoforms was negatively correlated with height SDS. Although the proportion of non-22-kDa GH isoforms in children with ISS was not significantly different from that in normal-stature children, 2 children with ISS had markedly elevated proportions of non-22-kDa GH isoforms (>20%), but in the same range as several girls with Turner syndrome [154]. Unfortunately, no specific clinical data on these children were presented, and further specification of the GH isoforms was not performed. As far as we know, the potential role of abnormal GH isoproteins in growth failure has not been studied thereafter. We conclude that the current understanding that the various GH isoproteins have a similar biological activity and the absence of any convincing case report makes it unlikely that an abnormal GH isoprotein profile may cause unexplained short stature.

Disturbances of GH1 Expression by Variants in the Promoter Region

As with any genetic disorder, clinical features of a syndrome cannot only be caused by a pathogenic variant in the coding sequence of a gene but also in the 5' or 3' regions around the gene, including promoter regions or enhancers. In a few studies, allelic variants in the GH1 promoter were studied, as a potential cause of decreased GH secretion.

Wagner et al. [155] analysed the GH1 promoter region for structural alterations and allelic variations in 113 patients with isolated GHD type 1B, 21 unaffected family members, and 78 normal-stature controls. Of the 22 sequence variation sites, 14% were located around the region of −1,075 bp, 77% between −550 bp and the translational start site (+1 bp), and 9% within the first intron. All the variations found in patients were also observed in non-affected family members as well as in normal unrelated controls. While these findings implied that there was not a single variation within the GH1 gene promoter, which causes isolated GHD, the authors could not exclude the possibility that combinations of variations might perturb expression.

The group from the Institute of Medical Genetics of the University of Wales College of Medicine did interesting work on the polymorphic variation in the proximal promoter and locus control region of GH1 [106, 156]. In a group of healthy male individuals, an association was noted between adult height and the mean in vitro expression value corresponding to an individual’s GH1 promoter haplotype combination, although it explained only 3.3% of the variance [156].

In the same year, Millar et al. [106] investigated GH1 variants, including GH1 proximal promoter haplotypes, in 41 individuals with short stature, reduced height velocity, and bone delay, as well as in 11 individuals with idiopathic GHD and 154 controls. For the purpose of the present review, we concentrate on short individuals with a normal result in the GHST, a variant in the GH1 promoter region, and a GH1 haplotype associated with a low expression level relative to the wt-haplotype. There were 3 individuals complying with these conditions. Cases 57 and 75 (with heights of −2.8 and −6.2 SDS and stimulated GH peaks of 27.3 and 6.8 μg/L, respectively) carried a heterozygous variant in the proximal promoter (−60G>A, recommended nomenclature according to [157]) associated with a haplotype with a low GH1 expression (haplotype 19 according to Horan et al. [156]), inherited from their mothers. However, the mothers of both cases had a fully normal height SDS (0.3 and −0.6 SDS), which makes it unlikely that the variant is causative for the short stature of the child. Case 76 (with a height SDS of −2.2 and a GH peak of 18.3 μg/L) carried a complex variant in the proximal promoter (−216A>G and −40_−39delGGinsCT), associated with haplotype 17 (associated with a low GH1 expression). The variant was not found in the parents, who both were short (−2.2 and −2.4 SDS), so also in this case a causative role of the variant in the patient’s short stature is unlikely.

In a Dutch study on 62 individuals with isolated GHD, several GH1 promoter SNPs were associated with height and IGF-I levels among patients and controls, but no data were reported on short individuals with low serum IGF-I and a normal GHST [158]. In conclusion, the available

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DOI: 10.1159/000516407

Horm Res Paediatr 2021;94:81–104
evidence does not support the hypothesis that disturbances of GHI1 expression by variants in the promoter region cause short stature in children with a low serum IGF-I and normal GH peak in a GHST.

Disturbances in the Translation of GHI1 mRNA
A disturbed GHI1 RNA translation was suggested in a study on 3 short children with normal or high GH secretion, normal GHBP serum levels, low IGF-I serum levels, catch-up growth under rhGH treatment, and absence of any GHI1 variant or anti-hGH antibodies [159]. Serum samples were measured by polyclonal hGH-RIA, Nb2 rat lymphoma proliferation assay, and GH immunofunctional assay. In comparison to controls, the patients’ serum GH levels were much lower when measured by Nb2 rat lymphoma cell proliferation bioassay and by the immunofunctional assay than by RIA. Retesting of 2 of the 3 patients including a 1 year break of therapy confirmed the rhGH dependence of growth in spite of normal endogenous GH secretion. The authors speculated that post-translational processing of GH might reduce the biological activity of the normal translation product.

**Conditions with Decreased GH Sensitivity**
In the following paragraphs, we shortly review the clinical features of the well-established causes of GHI characterized by low serum IGF-1 (pathogenic variants of GHR, STAT5B, STAT3, IGFI, and IGFALS) and 2 syndromes where partial GHI appears likely (Noonan and MIM #245590). There are additional, and characteristic, features: symptoms of immune dysfunction (e.g., severe eczema and chronic pulmonary disease) and elevated prolactin [27, 162].

Similarly to the continuous spectrum of GH secretion, there is also a continuous spectrum of severity of GH sensitivity. In addition, there is a large variability of the clinical presentation of individuals carrying pathogenic variants of GHR, STAT5B, STAT3, IGFI, and IGFALS. Therefore, any distinction between classical and non-classical forms, as previously proposed [27], is inevitably arbitrary. Still, for clinical purposes, such subclassification is useful, since the classical forms of these conditions, caused by a total functional loss of biological activity of the respective gene product, usually leads to such specific phenotype, that a candidate gene approach is warranted. Given the availability of recent reviews on these genetic disorders [27, 28, 161–164], we keep the descriptions of these conditions short.

**GHI due to GHR Defects**
The first reported disorder characterized by classical GHI was Laron syndrome (MIM #262500), caused by a complete biallelic defect of the gene encoding the GH receptor (GHR) [165]. Clinical features include extreme postnatal growth failure, midfacial hypoplasia, relatively normal head circumference, small external genitalia in males, sparse and thin hair, small hands and feet, delayed dentition and puberty, and hypoglycaemia. Biochemically, GH secretion is increased and serum concentrations of IGF-1, IGFBP-3, and ALS are severely subnormal and do not respond to an IGFGT. The serum concentration of GHBP is usually decreased but can be normal or even elevated depending on the position of the genetic variant [27, 161].

Non-classical GHI associated with GHR variants includes the GHR pseudoexon variant (6ψ) and heterozygous GHR variants. For some heterozygous GHR variants, a dominant-negative effect has been confirmed, but for many others, the clinical relevance is questionable [27].

**GHI due to STAT5B Defects**
Homozygous variants of STAT5B, first reported in 2003 [166], cause a similar pattern as Laron syndrome with regard to growth, bone age, pubertal delay, facial characteristics, and serum IGF-1, IGFBP-3, and ALS (MIM #245590). There are 2 additional, and characteristic, features: symptoms of immune dysfunction (e.g., severe eczema and chronic pulmonary disease) and elevated prolactin [27, 162].

A milder (“non-classical”) clinical presentation was found in 3 probands with heterozygous dominant-negative STAT5B variants and several of their relatives [167]. Heights ranged from 2.9 to –5.3 SDS and eczema and elevated IgE were noted, without severe immune or pulmonary problems. Elevated serum prolactin was only found in one of the 3 probands [27, 162, 167]. A similar clinical presentation was shown by 2 unrelated patients with a heterozygous STAT5B variant in combination with a heterozygous IGFALS variant [36, 168]. Other heterozygous STAT5B variants may also have some effect on growth and serum IGF-1, according to a study on heterozygous carriers of pathogenic STAT5B variants, without clinical features [169].

**GHI due to Activating Variants of STAT3**
Germline STAT3-activating (gain-of-function) variants result in early-onset multiorgan autoimmunity, lymphoproliferation, recurrent infections, and short stature
(MIM #615952). There are indications that such STAT3 variants may negatively regulate GH-induced STAT5B activation through the induction of SOCS3 protein, the formation of non-functional STAT3/STAT5 heterodimers, or through the competition for binding to target gene loci, activating different transcriptional programmes (reviewed in [163]). Milder forms with isolated growth failure have not been reported.

**GHI due to IGF1 Variants**

IGF1 variants can be categorized into biallelic loss-of-function variants, biallelic variants with decreased function, and heterozygous variants [28]. The classical presentation of children with a homozygous loss-of-function variant consists of extreme pre- and postnatal growth failure, poor feeding, severe microcephaly, retrognathia, sensorineural deafness, and severe global developmental delay (MIM #608747, reviewed in [28]). The first case with a deletion was reported in 1996 [170], followed by 2 cases with a missense variant [171, 172]. Cases carrying a biallelic IGF1 variant with decreased function have a milder phenotype, without hearing loss and developmental delay [173]. Serum IGF-I varies from undetectable to elevated, and IGFBP-3 and ALS are generally normal [28].

Heterozygosity for a pathogenic IGF1 variant or deletion can cause short stature [174–176] with a wide height SDS range. In 1 family, no dominant-negative effect of the truncated protein was shown [177]. A small effect of heterozygosity for a pathogenic IGF1 variant on growth and adult stature was also shown in relatives of patients with a homozygous IGF1 variant [171, 172, 178].

**GHI due to IGFALS Variants**

The characteristic clinical presentation of individuals carrying 2 pathogenic variants of IGFALS, first reported in 2004 [179], is mild-to-moderate short stature, delayed puberty, low serum IGF-1 and ALS SDS, and even lower IGFBP-3 SDS (MIM #615961). A low birth weight and/or length, reduced head circumference, and insulin resistance are commonly observed (for review, see [164]). Heterozygous carriers of an IGFALS variant frequently present with mild growth failure and subnormal levels of ALS, IGFBP-3, and IGF-1 [164, 180] and were reported in approximately 10% of children initially considered “idiopathic short stature” [164, 181].

**Unexplained Low Serum IGF-I in 2 Cases with Heterozygous IGF1R Variants**

As mentioned previously, the great majority of children carrying a heterozygous pathogenic IGF1R variant (MIM #270450) have a serum IGF-I in the upper half of the reference range or above [160]. However, at least 2 children presented with a decreased IGF-I concentration [182, 183], which has remained unexplained.

**Syndromes with Dysmorphic Features Associated with Diminished GH Sensitivity**

Partial GHI has been suggested for children with Noonan syndrome (MIM #163950) based on the observation of low IGF-I values, preserved GH secretion, and suboptimal growth response to rhGH therapy (reviewed in [184]). The precise mechanism is still unclear and may be associated with an increase in tyrosine phosphatase activity, since shp-2 binds to and dephosphorylates signalling molecules such as STAT5b [27]. Alternatively, activation of the RAS/MAPK pathway may play a role [184].

Three-M (3M) syndrome (MIM #273750) is associated with normal or high peak GH levels and normal or low IGF-I levels, and the growth response to rhGH is usually low. In vitro studies have provided evidence for a combined insensitivity to GH and IGF-1 [185].

**Abnormal Interaction of IGF-I with Its Chaperones**

Even if there is no abnormality of the coding region of the IGF1 gene nor in its expression and translation, the secretion of IGF-I may be decreased by other causes. One potential cause could be an insufficient functionality of the chaperone protein that is needed for a normal secretion of IGF-I and -II, that is, the chaperone glucose-regulated protein 94 (GRP94) [186]. Indeed, a hypomorphic variant (p.(Pro300Leu) variant NM_003299.2: c.962C>T p.(Pro321Leu) according to the HGVS nomenclature) was found in a child with primary IGF deficiency but was later considered a non-common SNP with frequencies of 1–4% in various populations [187]. Heterozygous carriers had a 9% lower circulating IGF-I concentration than non-carriers. When tested in the grp94(−/−) cell-based complementation assay, Pro300Leu supported only 58% of IGF secretion relative to wt-GRP94. Furthermore, re-combinant Pro300Leu showed impaired nucleotide-binding activity. The authors concluded that variants in GRP94 can affect its IGF-chaperone activity, which may represent a novel causal genetic mechanism that limits IGF biosynthesis [187], although this needs further confirmation.

**Therapeutic Options for Children with GHI**

The classical forms of GHI generally do not respond to rhGH treatment. For children with Laron syndrome, rhIGF is a registered treatment, leading to a modest in-
crease in height velocity and adult height [188]. There are few data on the effect of rhIGF in cases with homozygous or heterozygous STAT5B or IGF1 defects. Preliminary observations suggest that rhGH may have a positive effect in cases with heterozygous IGF1 variants [174].

While in children with biallelic IGFALS variants, rhGH and rhIGF appear ineffective, preliminary reports have suggested that rhGH could be effective to accelerate growth velocity in children who are heterozygous carriers of IGFALS variants [164, 189]. rhGH is registered for the treatment of Noonan syndrome, based on the moderate effect on height velocity and adult height [190]. The growth response to rhGH treatment in children with 3M syndrome is variable and modest [185]. Interestingly, GH treatment appears effective in increasing growth in girls with anorexia nervosa, despite the GHI associated with this condition [191].

**Hypothetical Causes of Short Stature Associated with a Low Serum IGF-I and Normal Stimulated GH Peak**

The current differential diagnosis is of course completely dependent on the presently available genetic toolkit in the clinic. However, in the meantime, new techniques have developed in basic science, which have not found their way to the clinic yet. One can expect that further technological advances will take place, which will help to get more insight into the complex signalling pathway of the GH-IGF-I axis. In the following paragraph, we speculate about the sort of clinical insights that may be generated using an intensified clinical use of presently available technology.

**Intensified Use of Exome and Genome Sequencing**

The past decades have shown an accelerating speed of discoveries of novel genetic causes of multiple congenital disorders, including disorders of the GH-IGF-I axis. There is little reason to think that this has reached the end.

Pituitary GH secretion is mainly controlled by GHRH and somatostatin [192], so one would expect that defects of the genes encoding these proteins or their receptors would cause abnormal GH secretion. Besides defects of the gene encoding the GHRH receptor, causing GHD type 1B, no genetic aberrations have been found in the genes encoding GHRH, somatostatin, and somatostatin receptors.

In theory, an activating variant in a somatostatin receptor expressed in the pituitary might cause a decrease in GH secretion, serum IGF-I, and height, and the result of a GHST acting through GHRH activation could be normal. Four out of the total of 5 somatostatin receptors are expressed in the pituitary, but predominantly the receptors encoded by SSTR2 and SSTR5. Interestingly, in a study on patients with acromegaly and controls, 2 polymorphisms in SSTR5 were associated with serum IGF-I and IGFBP-3 [193]. Another SSTR5 variant was associated with 11% lower levels of circulating IGF-I and IGFBP-3 [194]. We speculate that genetic variants will be discovered in somatostatin receptors or other components of the complex GH regulatory system, which may cause short stature associated with low IGF-I and a normal GHST result.

*Potential Yield of Studies on Cellular Pituitary Crosstalk (“Paracrinicity”)*

So far, the publications on cellular pituitary crosstalk have only reached the eyes of few clinical endocrinologists. However, these studies have generated very interesting information that theoretically may be associated with clinical phenotypes. To cite one of the key investigators in this field, Dr. C. Denef from Louvain (Belgium): “in the anterior pituitary, paracrine communication and autocrine loops that operate during foetal and postnatal development in mammals and lower vertebrates have been shown in all hormonal cell types and in folliculo-stellate cells. More than 100 compounds have been identified that have, or may have, paracrine or autocrine actions” [195].

We believe that the studies on the induction of functional hypothalamus and pituitary tissues from pluripotent stem cells, which may result in an “artificial pituitary” [196], may generate novel insights in the complex cellular intra-pituitary interactions. This may also lead to novel defects of GH secretion that have still remained in hiding.

An example of a condition that may be associated with abnormal cellular pituitary crosstalk is the IGSF1 deficiency syndrome (MIM #300888, caused by a hemizygous defect of IGSF1 [197]), in view of decreased Igsf1 expression in the somatotroph, lactotroph, and thyrotrhop cells in the rat [198] and variable deficiency of the respective pituitary hormones in humans. Partial and transient GHD is encountered in approximately 10% of males with IGSF1 deficiency syndrome, while GH secretion tends to increase above the normal range in adults [199].
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Potential Yield of Tools to Estimate DNA Methylation Status and Histone Modification
Imprinting Disorders and Other Methylation Disturbances
The best known imprinting disorders, for example, uniparental disomy and other methylation disturbances, associated with short stature include Silver-Russell syndrome, Temple syndrome, IMAGe syndrome, and Prader-Willi syndrome. Three out of the 5 forms of Silver-Russell syndrome (MIM #180860, #618905, and #616489) are known to be caused by an imprinting disorder [200]. The 40% of patients with the clinical features of Silver-Russell syndrome in whom no (epi-)genetic cause can be found with current technology suggest that other forms may be detected in the future, although these patients usually show normal or slightly elevated IGF-I. The GH-IGF-I axis in Temple syndrome (MIM #616222) and IMAGe syndrome (MIM #614732) has not been investigated in depth. So far, there is little indication that this axis is affected, except for 1 case with IMAGe syndrome with a low GH peak [201]. In short children born SGA, several DNA methylation changes at multiple loci were observed [202, 203], but the potential association with the GH-IGF-I axis remains to be established.

Histone Modification
The expression of genes is not only regulated by methylation status but also by histone modification. There are a number of genes encoding enzymes that catalyse post-translational histone modifications, such as methyltransferases, demethylases, acyltransferases, chromodomain helicases, and arginine-methyltransferases. An indication that abnormal modification may play a role in growth regulation is offered by the observation that pathogenic variants of KDM3B cause intellectual disability, short stature, and facial dysmorphism. KDM3B encodes a histone demethylase and is involved in H3K9 demethylation, a crucial part of chromatin modification required for transcriptional regulation [204]. A better insight in histone physiology may lead to novel tests for aberrations of histone functionality and possibly novel syndromes associated with low IGF-I and normal GH secretion.

Potential Yield of RNA Sequencing
RNA sequencing is available in the laboratory but has rarely been used in the clinic. Still, RNA sequencing has helped in re-evaluating and further classifying the genetic variants found by exome sequencing, such as confirmation of putative splicing mutations [205]. It is also used to determine whether 2 variants in the same gene are localized on the same or different chromosome and to detect monogenic defects undetected by exome sequencing (e.g., a deep intronic variant leading to a pseudoexon) [206, 207].

RNA sequencing can also be targeted to analyse long non-coding RNAs (lncRNA). lncRNAs are transcripts of >200 nucleotides in length not containing an extended open reading frame; 28,000 lncRNAs are annotated in the human genome. Defects have been associated with a number of diseases, including Silver-Russell syndrome (H19), Temple syndrome, cartilage-hair hypoplasia, and Turner syndrome (XIST) [208].

Specific PCR tests have been developed for micro-RNAs (miRNA expression profiles). miRNAs are epigenetic regulators of gene expression that act at the post-transcriptional level, influencing regulatory gene networks [209]. Several miRNAs regulate the growth plate and GH-IGF axis, contributing to longitudinal growth. For example, miR-709 inhibits GHRP6-induced GH synthesis by targeting PRKCA in the pituitary [209, 210]. There are indications that miRNAs are also involved in catch-up growth in children born SGA [211].

General Conclusions
The differential diagnosis of a non-syndromic short child with low circulating IGF-I and a normal GH peak in a stimulation test is extensive. Numerical data are not available, but our impression is that the major causes are discordance between stimulated and spontaneous GH secretion and partial GHI (including Noonan syndrome, which can present with few dysmorphic features). Of the genetic conditions associated with normal GH sensitivity, bioinactive GH (Kowarski syndrome), is well documented, while there is still doubt about the role of GHSR variants.

We believe that genetic assessment of such patients is indicated, given that for cases with classical GHI, such as Laron syndrome and biallelic STAT5B variants, GH treatment is not warranted. Instead, such patients are candidates for rhIGF treatment. However, various other genetic disorders are expected to respond well to rhGH treatment, such as heterozygous carriers of IGF1 or IGFALS variants.

For an effective diagnosis of such patients as well as other patients suspected for one of the many genetic disorders associated with short stature, the establishment of a multidisciplinary team on growth genetics has proven to be very beneficial, as well as a joint clinic of a paediatric endocrinologist and clinical geneticist. A challenge for the future is how to deal with previous patients suspected...
for a genetic aetiology with initially negative genetic findings. We believe that a guideline is needed on the selection of such patients who may be called back to the clinic when new genetic tools become available in future years. The potential advantage of such an approach is illustrated by the observation that re-analysis of exome data of children with developmental disorders increased the diagnostic yield from 27 to 40% [212].

Acknowledgements

This minireview was inspired by, and is a tribute to, Dr. Marie-José E. Walenkamp, who played a crucial role in developing the IGFGT and in unravelling the genetic aetiologies of short children. Marie-José Walenkamp was planned to co-author this minireview, but unfortunately, this was made impossible because of a sudden devastating disease. She sadly passed away in March 2021, much too young. We are grateful to Drs. Kerstin Albertsson-Wikland and Berit Kristrom for helpful advice on the section on spontaneous GH secretion and for kind consent to present Figure 1.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

Funding Sources

The authors did not receive any funding.

Author Contributions

J.M.W. initiated this review, reviewed the literature, and took the lead in writing the text. S.D.J., M.L., H.A.D., and C.B. revised previous versions of the manuscript. M.L. and H.A.D. revised the tables and the text on genetic issues. All authors read and approved the submitted manuscript.

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