The Challenge of Defining and Investigating the Causes of Idiopathic Short Stature and Finding an Effective Therapy

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Keywords

Growth hormone · Idiopathic short stature · Insulin-like growth factor-1 · Short normal children

Abstract

Idiopathic short stature (ISS) comprises a wide range of conditions associated with short stature that elude the conventional diagnostic work-up and are often caused by still largely unknown genetic variants. In the last decade, the improvement of diagnostic techniques has led to the discovery of causal mutations in genes involved in the function of the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis as well as in growth plate physiology. However, many cases of ISS remain idiopathic. In the future, the more frequent identification of the underlying causes will allow a better stratification of subjects and offer a tailored management. GH therapy has been proposed and approved in some countries for the treatment of children with ISS. To improve the efficacy of GH therapy, trials with GH combined with GnRH agonists, aromatase inhibitors, and even IGF-I have been conducted. This review aims to revise the current definition of ISS and discuss the management of children with ISS on the basis of the most recent evidence.

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Introduction and Definition of Idiopathic Short Stature

Short stature in childhood is the most common reason for referral to pediatric endocrinologists. The majority of short children, accordingly to the internationally accepted definition, may be labeled as having idiopathic short stature (ISS) [1]. ISS is currently defined as the presence of a height more than 2 SD below the corresponding mean height for a given age, sex, and population in a child with a normal birth size and normal body proportions and without evidence of any systemic, endocrine, nutritional, or chromosomal abnormalities [2].

ISS children may present with a family history of shortness and have a stature below the normal but within the parental target range (familial ISS) or they have a height below the familial target range (nonfamilial ISS). A delayed skeletal maturation and a delayed onset of pubertal development may also be present [2]. Consistent with this widely accepted definition, ISS includes a heterogeneous group of children with variable phenotypes and genotypes.

Classically, most subjects referred for short stature have “normal variants of growth,” such as familial short stature (FSS; healthy short subjects with normal maturation).

tion and a family history of short stature) or constitutional delay of growth and puberty (CDGP; healthy subjects with slow maturation and delayed bone age and start of puberty), both characterized by the achievement of an adult height within the familial target range. Therefore, the current definition of ISS includes short children with normal variants of growth as well as children with pathological conditions severely affecting their growth outcome, such as unrecognized genetic defects of the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis and mild forms of bone/cartilage dysplasia. This extreme heterogeneity of the ISS population inevitably affects the response to GH treatment as well as to the other therapies proposed so far. It is our conviction that children with CDGP should be excluded by the definition of ISS and, consequently, not be considered for GH therapy under this diagnostic category. Children with CDGP achieve, though with delay, a normal adult height and are not candidates for remaining permanently short.

Regarding FSS, it refers to a form of short stature with a heterogeneous etiology and which is present in at least 1 short parent/relative. The evaluation of these subjects should carefully consider the family history and the severity of the shortness. In milder forms a polygenic involvement can be hypothesized, whereas in cases of severe FSS a monogenetic underlying cause should be suspected [3]. Consistently, a recent paper conducted on small-for-gestation-age (SGA) or GH-deficient (GHD) subjects with familial shortness showed that monogenic conditions could be detected in up to 52% of the subjects with severe familial shortness [4].

Shortness is a multifactorial condition regulated by genetic, epigenetic, and environmental factors [3]. By applying different genetic techniques such as copy number variant (CNV) analysis, the single gene approach, or whole-exome sequencing (WES), approximately 25–40% of children diagnosed with ISS receive a molecular diagnosis [5]. Specific genetic defects have been associated with ISS, such as mutations of genes like SHOX, aggrecan (ACAN), fibroblast growth factor receptor 3 (FGFR3), natriuretic peptide receptor 2 (NPR2), or components of the GH/IGF-I axis [6]. One of the major challenges in the management of ISS children is the identification of subjects deserving genetic testing, as clinical signs and symptoms are often not suggestive of a specific genetic defect. A molecular diagnosis is important as it may potentially drive treatment decisions, favor a tailored management, and offer adequate genetic counseling. Systematic phenotyping combined with targeted genetic testing and exome sequencing increases the diagnostic power in short stat-

ure, and in the future a thorough molecular characterization will likely allow subcategorization of subjects previously labeled as ISS. Although ISS subjects are not GHD, some of them show subnormal circulating levels of IGF-I [7–9].

Nevertheless, ISS children, by definition, have normal GH responses to stimulation tests, although other alterations of the GH/IGF-I axis have to be excluded [10]. Mild forms of GH resistance may be involved in some ISS children, although a recent paper showed no significant differences in the response to an IGF-I generation test between ISS and sex- and age-matched normal stature children [11].

This minireview aims to revise the current definition of ISS and discuss the management of children with ISS on the basis of the most recent evidence.

Diagnostic Approach

ISS is an exclusion diagnosis that is achieved after ruling out other recognizable causes of short stature.

The complex diagnostic work-up of children with short stature includes a thorough history and physical examination, evaluation of the growth trajectory (compared with mid-parental height) and height velocity, assessment of skeletal maturation, and eventually ad hoc laboratory investigations [1, 12]. Typically, investigations aim to rule out hormonal dysfunctions or chronic diseases affecting growth. Figure 1 reports the current simplified and practical diagnostic work-up based on the clinical features of a short child in order to avoid unnecessary and often misleading tests that may lead to costly, useless, and potentially harmful therapies.

Recently, the importance of a careful assessment of body proportions and the need to consider the diagnosis of skeletal dysplasia in subjects with ISS has been highlighted [13]. Indeed, signs of skeletal dysplasia were found in up to 22% of ISS patients [13], with the prevalence reaching 33% when a parent was also affected.

After excluding other conditions, a major challenge in the management of a child with ISS is the decision of whether or not to perform genetic testing. In fact, although most cases have no specific diagnosis, in a high percentage of subjects a genetic cause is conceivable; however, at the moment, there is no consensus about the best genetic work-up for defining short stature as idiopathic.

Height is a polygenic trait influenced by several genes. Approximately 80–90% of adult height is heritable [14] and, so far, genome-wide association studies have identi-

Fig. 1. Diagnostic algorithm for the management of a short child.

fied a wide range of associated genetic loci, either involved in cellular or metabolic mechanisms or related to growth-regulating systems or that are important for the growth plate [15, 16]. Overall, these gene variants explain about 30% of adult height variations [5]. Genetic variants have various influences on height. Common variants individually have a minimal effect on height but a major impact on growth in the case of coexistence [17–20]. By contrast, in some cases of ISS shortness is related to rarer heterozygous mutations in genes previously implicated in autosomal-recessive skeletal dysplasias (ACAN and NPR2), confirming a dosage effect of cartilage matrix proteins in growth [6]. Therefore, several studies have used a candidate gene approach to detect genes involved in growth disorders, but this approach is characterized by a low yield and identifies the underlying cause of shortness in less than 5% of subjects. One of the main challenges lies in the lack of a suggestive clinical phenotype addressing toward a specific etiology. In these cases, a multigene approach looks more promising and the recent increased use of genome-wide approaches is definitely changing the diagnostic approach to shortness.

Monogenic Causes of ISS

GH/IGF-I Axis

The GH/IGF-I axis is the most important signaling pathway regulating growth. Interestingly, whereas homozygous or compound heterozygous mutations of the GH receptor (GHR) are responsible for complete GH insensitivity, mild GHR gene mutations may cause ISS, characterized by low IGF-I serum levels and high GH levels [21]. A recent study has identified variants in the GHR gene region in subjects with ISS [22], and a heterozygous mutation in the extracellular domain of the *GHR* gene was found in 4 out of 14 ISS children with low levels of GH-binding proteins [23]. Mild GHR mutations have been proposed to account for approximately 5% of ISS subjects [21, 24]. Abnormal GHR signaling may also be implicated in ISS [25, 26] and IGF-I haploinsufficiency has been detected in an ISS subject with IUGR, short stature, microcephaly, and cognitive delay [27]. Additionally, specific single-nucleotide polymorphisms in the IGF-I receptor (IGF-IR) gene are associated with genetic susceptibility to short stature [28]. Heterozygous *IGFALS* gene mutations have been found in a subgroup of ISS

children with reduced IGF-I and IGFBP-3 levels and partial acid-labile subunit deficiency [29]. Pregnancy-associated plasma protein A2 (*PAPP-A2*) is a protease that, by fragmenting IGFBPs and thus reducing their affinity for IGFs, ultimately leads to increased IGF-I bioavailability. Recently, a new condition characterized by a mutation of *PAPP-A2* has been described in members of two unrelated families with an unexplained severe short stature and high circulating levels of GH, IGF-I, IGF-II, IGFBP-3, and IGFBP-5 and no detectable or low levels of pregnancy-associated plasma protein A2 (*PAPP-A2*) [30]. Future studies will assess the frequency of *PAPP-A2*-mutations in ISS children.

Independently of the GH-IGF-I system, many other factors involved in the regulation of paracrine signals, hormonal regulation, extracellular matrix, signaling pathways, and cellular functions influence growth [31, 32].

SHOX Gene

One of the best described growth genes in humans is the short stature homeobox-containing (*SHOX*) gene, reported in 1997 as a potential genetic cause of ISS [33]. *SHOX* is located in the short-arm pseudoautosomal region (PAR1) of X and Y chromosomes. *SHOX* haploinsufficiency and underlying syndromic and nonsyndromic short stature follow an autosomal dominant inheritance pattern [34] and its mutation causes a broad phenotypic spectrum with an apparent gene-dose effect [32].

SHOX haploinsufficiency is caused more frequently by CNVs than by point mutations [34], and it has been found in approximately 70% of patients with Leri-Weill dyschondrosteosis and in subjects with Madelung deformity, a combination of anatomical changes in the wrist [35, 36]. *SHOX* haploinsufficiency is responsible for shortness in Turner syndrome patients [33] and, notably, *SHOX* mutations may account for about 2–4% of ISS patients [37]. A clinical score has been developed to predict the likelihood of a *SHOX* defect and identify patients who are suitable for genetic analysis [38].

FGFR3 Gene

FGFR3 is a negative regulator of growth plate chondrogenesis [39], whose activating mutations result in inhibited long-bone growth and are responsible for achondroplasia. Minor defects of the *FGFR3* gene are responsible for milder clinical phenotypes, such as hypochondroplasia, sometimes hardly distinguishable from ISS. By evaluating the prevalence of *FGFR3* gene altera-

tions among 54 individuals with an ISS diagnosis, no pathological mutations were found, suggesting that *FGFR3* polymorphisms play a marginal role in influencing stature in normal individuals [40]. This is in contrast with the observation that a proportionate short stature can be caused by *FGFR3* mutation [41].

ACAN Gene

ACAN, encoded by the *ACAN* gene, is the most abundant proteoglycan of the growth plate cartilage, and it is a newly recognized important factor for cartilage and bone morphogenesis. *ACAN* gene mutation leads to an abnormal structure of the cartilage extracellular matrix, reduced chondrocyte proliferation, and accelerated hypertrophic chondrocyte differentiation. *ACAN* mutations can lead to a wide clinical spectrum ranging from spondyloepimetaphyseal dysplasia, characterized by severe short stature, brachydactyly, and midface hypoplasia to milder skeletal dysplasia, associated with a variably compromised adult height. Recently, ISS has been associated with *ACAN* haploinsufficiency [42] and heterozygous defects in *ACAN* have been recognized as a cause of ISS (present in 1.4% of patients with ISS) next only to *SHOX* defects [43]. In a recent study, next-generation sequencing (NGS)-based mutation screening of known causative genes of both skeletal dysplasias and abnormalities in the GH-IGF-I axis was performed in 86 patients with ISS. The results of that study showed that *ACAN* mutations can be responsible for ISS in subjects without characteristic skeletal dysmorphisms whereas mutations in *FGFR3*, *NPR2*, and GH-IGF-I axis genes play a marginal role in ISS [6].

NPR2 Gene

Heterozygous loss-of-function mutations of the *NPR2* gene, encoding for the receptor of c-type natriuretic peptide which binds the c-type natriuretic peptide and is encoded by natriuretic peptide precursor-C (NPPC), are responsible for short stature in patients without a distinct phenotype. *NPR2* and c-type natriuretic peptide have been recently identified as regulators of chondrocyte development and growth [44] and genome-wide association studies have highlighted the involvement of this system in influencing height variation [45]. Probably, the effect of the *NPR2*/c-type natriuretic peptide system on growth is due to its influence on *FGFR3* signaling [46]. *NPR2* mutations have been related to different clinical phenotypes ranging from skeletal dysplasia to ISS and *NPR2* mutations have been found in up to 6% of children with ISS [45]. Nevertheless, the role of *NPR2* in ISS is still

unclear as its variants were not found in 86 unrelated Japanese ISS patients [6]. A recent study on 697 patients with disproportionate or proportionate short stature, found a heterozygous mutation in the NPPC gene in 2 families with proportionate short stature, small hands, and mild facial abnormalities [47].

Chromosomal CNVs

By using hybridization of a patient's DNA and reference DNA, chromosomal microarray detects genomic imbalances such as submicroscopic deletions and duplications. These imbalances are known as CNVs and include either heritable or novel variations in the number of gene copies, involving one or more genes functionally implicated in growth regulation. CNVs seem to be responsible for approximately 10% of ISS cases [14], with a higher rate in cases with additional malformations. A recent study performed in 162 patients belonging to 149 unrelated families showed a lower rate of CNVs associated with a short stature, 6 CNVs in 6 families (4%) were certainly associated with a short stature whereas 40 CNVs in 33 families (22.1%) were considered as possibly pathogenic [48]. Consistently, in 119 Chinese children with ISS, 5 pathogenic or possibly pathogenic CNVs were detected in 5 patients. Taking only the certain pathogenic variants into account, the diagnostic yield was 2.5% (3/119) [49].

In a further study performed in 19 patients with a height below -2 SD, a total of 61 CNVs not previously described as normal variants in a database of genomic variations was found but only 3 out of 19 subjects had CNVs containing genes related to a short stature [50].

The knowledge stemming from the identification of CNVs in children with ISS can help to identify novel genes involved in growth regulation and in the pathophysiology of ISS.

Next-Generation Sequencing

The availability of the genome-wide approach has led to the detection of pathological variants in children with ISS as well as to the discovery of novel genes and pathways involved in growth regulation. The major limitation of a multi-gene approach is represented by the possibility of finding variants of unknown significance and the difficulty of interpretation. The use of WES has the advantage of analyzing the coding DNA and therefore of considering a more limited amount of data.

In 2014, Guo et al. [51] showed that in a cohort of 14 well-characterized patients with undefined severe short

stature the diagnostic yield of WES was 36%. Further studies have confirmed the utility of this approach despite variable results. A diagnostic yield of 16.5% of exome sequencing in patients with isolated short stature [52], which rose to 21% in cases with associated syndromic features, has been reported.

NGS has recently been proposed as a primary diagnostic tool in the work-up of children with ISS. In a cohort of 114 Chinese children with ISS, the potential genetic cause was identified in 41 patients (36%) and most cases were detected by NGS (33.3%) [53]. As facial dysmorphism and/or skeletal abnormalities were associated with a significantly higher diagnostic rate, these features may represent clues for specific genetic evaluation. Interestingly, NGS was applied to a cohort of 33 children with severe FSS treated with GH for SGA or GHD indications [4]. This approach led to the identification of an underlying genetic cause in half of the patients (52%), with a high prevalence of growth plate single-gene variants, confirming the need for a genetic approach in cases of severe FSS.

Final Remarks

Epigenetic changes are also involved in the regulation of growth [54], so that imprinting disorders should also be considered when approaching a child with ISS. Common variations may affect human growth by parental imprinting in humans [19].

Overall these data show that many children labeled as having ISS may have a defined genetic cause and that different genes should be considered in the differential diagnosis of ISS. At least until exome (or genome) sequencing is available at costs so low as to allow its large-scale use in ISS children, it would be suitable to perform genetic testing in subjects who are highly clinical suspicious for genetic defects. Table 1 summarizes some genes that have been detected in ISS subjects.

Therapeutic Management

ISS subjects reach an average final height of -1.5 SD in boys and -1.6 SD in girls [55, 56], achieving an adult height usually within the target height in familial ISS and of approximately 0.5 SD below the target height in non-familial ISS. However, these findings refer to the broad definition of ISS, being derived from studies conducted on subjects with both FSS and CDGP who represent the vast majority of children labelled as ISS. Ruling out CDGP cases, the adult height of more selected patients would presumably be shorter. Additionally, the extreme heterogeneity of the ISS population makes it difficult to draw conclusions about the efficacy of the different available

Table 1. Main genetic alterations reported in ISS subjects

Gene	Location	Pathologies	Estimated prevalence in ISS	Reference
<i>GHR</i>	Short arm of chromosome 5 between positions 13.1 and 12	Laron dwarfism	5%	21, 24
<i>IGF-I</i>	Long arm of chromosome 12 at position 23.2	IGF-I deficiency	Case reports of IGF-I haploinsufficiency, marginal role	27
<i>IGFIR</i>	Long arm of chromosome 15 at position 26.3	IGF-I resistance	Not estimated	28
<i>IGFALS</i>	Short arm of chromosome 16 at position 13.3	Acid-labile subunit deficiency	Unclear	29
<i>PAPPA2</i>	Long arm of chromosome 1 at position 25.2	Syndrome characterized by severe short stature, high levels of IGF-I, IGF-II, IGFBP-3, and IGFBP-5	Not estimated	30
<i>SHOX</i>	Short arm pseudoautosomal region (PAR1) of X and Y chromosomes	Leri-Weill dyschondrosteosis, Madelung deformity, Turner syndrome	2–4%	37
<i>ACAN</i>	Long arm of chromosome 15 at position 26.1	Wide clinical spectrum of skeletal dysplasia	1.4%	43
<i>NPR2</i>	Short arm of chromosome 9 at position 13.3	Skeletal dysplasia	Unclear	45
<i>FGFR3</i>	Short arm of chromosome 4 at position 16.3	Achondroplasia, hypochondroplasia	Marginal role	40

therapeutic approaches on growth. Hopefully, future studies will consider a more precise stratification of these subjects and hence provide clearer data. Considering these limitations, several therapeutic trials have been performed to increase height in ISS subjects.

GH Therapy

Efficacy. GH has been extensively used in the treatment of children with ISS since the first short-term trials published in the 1980s and early 1990s [57–63]. In 2003, GH therapy was approved in the USA for ISS children with a height at or greater than 2.25 SD (1.2 centiles) below the mean for their age and sex, associated with growth rates unlikely to permit attainment of an adult height in the normal range, and in whom a diagnostic evaluation excluded other causes for a short stature that should be observed or treated by other means. The recommended daily dose of rhGH is higher (i.e. 45 µg/kg of body weight) than the dose used in GHD, being based on the possible impaired sensitivity to GH in ISS patients. The first-year response seems particularly relevant for predicting the long-term response and a height gain below –1 SD in the first 12 months of rhGH treatment is considered inadequate [15]. Nevertheless, a consensus on the definition of an inadequate first-year GH response is still lacking and probably parameters such as age and underlying diagnosis should be considered to define the kind of response [64].

In an attempt to predict the growth response to therapy, an equation containing 4 predictive parameters (height at GH therapy start, height velocity during the first year of treatment, age at GH therapy start, and mid-parent height) has been developed and explains 64% of the variability of adult height, with an error of 0.63 SD [65].

We carried out a systematic review and meta-analysis of the available evidence supporting the indication of rhGH in children with ISS. The results of this meta-analysis showed that only 3 randomized controlled trials (overall including a total number of 115 children) were conducted from 1985 to April 2010. The evaluation of long-term efficacy showed that the adult height of the GH-treated children exceeded that of the controls by 0.65 SD (about 4 cm). In the 7 nonrandomized controlled trials the adult height of the GH-treated group exceeded that of the controls by 0.45 SD (~3 cm) [66]. Our conclusions were that GH therapy in children with ISS seems to be effective in partially reducing the deficit in height as adults, although the magnitude of the effectiveness is on average less than that achieved in other conditions for which GH is licensed. Furthermore, the individual response to therapy is highly variable, and additional studies would be needed to identify the responders.

In 2016, new guidelines for rhGH treatment were released by the Pediatric Endocrine Society. According to these guidelines, the decision to treat a child with a height below –2.25 SD has to be evaluated on a case by case basis,

carefully considering physical and psychological aspects as well as risks and benefits. The choice to start or not should be shared with the family [67], informing them that the average increase in height is approximately 5 cm and that the response is highly individually variable. The variability of the response needs careful monitoring of the first-year growth response and prediction models of the long-term response may be helpful in driving the management. However, no consensus was achieved about the definition of an adequate first-year growth response that is positively influenced by a younger age at start, a more delayed bone maturation, and a taller mid-parental height [68]. The starting rhGH dose proposed was 0.24 mg/kg/week, though in some cases a dose up to 0.47 mg/kg/week was considered appropriate. The efficacy of GH treatment in ISS children has been questioned as this therapy, while increasing the height gain during treatment, also accelerates bone maturation and leads to similar adult heights in treated and untreated subjects [69].

GH Therapy

Safety. The issue of rhGH safety has been extensively investigated in recent years, with a particular focus on cancer risk. In 2012, a study conducted in a French cohort of about 6,500 young adults treated with rhGH during childhood for GHD, ISS, or SGA found an increased risk of mortality for bone and cartilage cancer and cerebrovascular accidents [70]. In contrast to the French data, another study involving approximately 2,500 patients with the same diagnoses from Belgium, Sweden, and The Netherlands reported not a single case of death from cancer or cerebrovascular disease [71]. Both studies had a series of potential biases affecting the results, such as the relatively small sample size, the low event rate, and the lack of an untreated control group available for comparison, which is an unavoidable weakness. The opposite results may be due to differences in genetics or environment exposure or, more simply, to confounding factors [72].

In a subsequent morbidity study investigating the incidence of stroke and stroke subtypes in a population-based cohort of 6,874 patients in France treated with GH for short stature in childhood, a higher risk of hemorrhagic stroke and particularly subarachnoid hemorrhage was reported [73].

A survey conducted in 3,847 Swedish patients showed no increase in mortality in childhood rhGH-treated GHD, ISS, or SGA patients when applying an advanced gender-specific mortality model adjusting for birth characteristics [74].

More recently, results from the European Safety and Appropriateness of GH treatment in Europe (SAGhE) have been reported. The cohort for cancer mortality risk analyses comprised 23,984 patients and that for cancer incidence comprised 10,406 patients; the average follow-up length for mortality was 16.5 years and it was 14.8 years for cancer incidence (morbidity). The mean age at the end of follow-up was 27.1 years for the cancer mortality analyses and 25.8 years for the incidence analyses. That study showed no increased risk of mortality and morbidity for cancer in patients with an initial diagnosis of GHD, ISS, and SGA [75]. Finally, a population-based cohort study in France recently reported an increased risk of bone tumors after GH treatment in childhood for an isolated GHD, a short stature associated with a low birth weight or length, or ISS [76].

The described divergent results leave open the issue of the long-term safety of GH therapy. However, most findings argue against a major risk of cancer within the length of follow-up currently available, especially for children without other predisposing conditions. Nevertheless, continued vigilance during follow-up is desirable, both because of the lack of data for a longer follow-up and because of the presence of some significant raised risks in the results published so far.

Alternative Therapeutic Options

CDGP is often confused with ISS and boys with ISS may have a delayed pubertal development. Short-term administration of testosterone has been proven to be effective in encouraging the onset of puberty in boys with CDGP without substantially affecting the adult height [77–83]. Androgen treatment has also been considered among the treatment options for ISS boys who have a delayed pubertal onset.

Another therapeutic possibility is the use of a testosterone analog (oxandrolone), which is an androgenic steroid with less androgenic effects than testosterone and which is not aromatized into estrogen. This drug seems to increase the height velocity in children with a growth delay without influencing their adult height.

Gonadotropin-Releasing Hormone Analogs

The rationale of increasing height by slowing bone maturation and pubertal progression, thus prolonging the time available for growth, has supported the use of gonadotropin-releasing hormone analogs (GnRHa) in children with ISS [84]. In 1993, preliminary results about the effect of a 4-year GnRHa treatment in 16 children with short stature and normally timed puberty were reported [85].

An increase of 10.9 cm in the predicted adult height was observed in the treated patients compared to the placebo-treated patients. Nevertheless, data regarding the adult height were not available. In a subsequent study, GnRHa was used for an average of 23 months in 31 girls with constitutional short stature and recent onset of puberty [86]. The therapy was worthless as it increased the final height by 1 cm, and thus this treatment was not justified to stimulate growth. A randomized clinical trial was performed in 50 (24 subjects with a diagnosis of ISS and 26 subjects with a specific cause of short stature) adolescents who received either placebo or GnRHa treatment for 3.5 years and were followed up until the achievement of an adult height [84]. The GnRHa treatment led to a modest gain of 4.2 cm in adult height both in the ISS group and in subjects with recognized growth-limiting syndromes. Impaired bone mineral density was observed in the treated group.

Overall, the available evidence does not support the sole use of GnRHa in children with ISS and normally timed puberty [87], also considering that GnRHa therapy may lead to increased BMI and possible psychological consequences due to delayed puberty [77, 84, 88]. Additionally, GnRHa therapy may negatively affect bone mineral density, but long-term follow-up studies are lacking.

GnRHa and rhGH

The efficacy of a combined treatment of GnRHa and rhGH has also been tested as a potential therapeutic option, though with extremely variable results.

The combined therapy was used for a period ranging from 24 to 36 months in 10 short girls with a normal onset of puberty [89]. After 12 months of treatment, an improvement of the predicted height was observed and persisted when the treatment was withdrawn. The adult height did not differ from the predicted height at the beginning of the treatment. A randomized controlled study showed that a 3-year therapy with combined rhGH and GnRHa led to a significant increase of 8.0 cm in girls and 10.4 cm in boys over the predicted adult height at baseline, without side effects [90]. Unfortunately, adult height data were not available.

The efficacy of rhGH and GnRHa combined treatment on adult height was evaluated in 12 girls with ISS and normal or early puberty [91]. Results were compared with those of a comparable group of 12 girls treated with GH alone. Patients treated with rhGH plus GnRHa showed an adult height significantly higher than the pretreatment predicted adult height, with a mean height gain of 10 cm which is higher than that associated with rhGH treatment alone. Very recently, a retrospective observational study reported

the efficacy of GH + GnRHa association in 192 ISS subjects treated either with GH alone (70%) or with the combined therapy (30%) [92]. The combined treatment was effective in improving adult height compared to the predicted adult and target heights, with a higher efficacy in children who were prepubertal at the beginning of the GH therapy. However, most children, independently of the treatment regimen, reached an adult height within their mid-parental height, suggesting that most of the enrolled children had a CDGP [92]. By contrast, another study showed a modest efficacy of the combined treatment on adult height. A cohort of 17 short adolescents receiving the combined rhGH + GnRHa treatment was compared with 15 girls receiving no therapy. Adult height was not different between treated and control groups [93] and the combined therapy led to a net height gain of 4.9 cm. Remarkably, a tendency toward lower lumbar spine bone mineral density and bone mineral density was noted in treated boys.

Finally, a recent prospective randomized study was performed in 88 ISS subjects treated with rhGH alone or rhGH + GnRHa [94]. A premature interruption after approximately 2.4 years due to the regulatory authority request prevented to evaluate the effect of the combined therapy on near final height, but the available data indicated that the overall height gain was similar for patients treated with or without GnRHa. Interestingly, safety concerns emerged as a reduced mean total body BMC and a higher frequency of bone fractures were found in subjects receiving the combined therapy, suggesting a possible deleterious effect on bone.

On the basis of these findings showing a variable but overall poor efficacy and possible adverse effects on bone, the combined treatment of GnRHa and rhGH is not currently advised for treating ISS children [87].

Aromatase Inhibitors

Estrogens promote and accelerate bone maturation by inducing depletion of progenitor cells in the resting zone, accelerating the programmed senescence of the growth plate, and causing earlier proliferative exhaustion [95, 96]. Therefore, the inhibition of aromatase that converts androgens into estrogens has been proposed for treating children with various growth disorders.

The efficacy of aromatase inhibitors (AI) in improving the predicted adult height was first observed in patients with CDGP. In 23 boys with CDGP receiving testosterone + placebo or testosterone + letrozole, the predicted adult height increase of 5.1 cm was observed in patients treated with letrozole while no change was found in patients who received placebo [97]. Although the need to use AI to

Table 2. Therapeutic approaches performed in ISS subjects

Treatment	Study population	Design	Efficacy	Comment	Reference
rhGH	3 randomized controlled trials (115 children)	Meta-analysis	AH increase in GH-treated patients: 4 cm	Extreme variability of the response	66
GnRHa	(a) 50 adolescents (24 with ISS and 26 with a specific cause of SS)	(a) Randomized, double-blind, controlled trial	(a) Gain of 4.2 cm in AH	(a) Duration of treatment: 3.5±0.9 years and decreased accretion of bone mineral density	84
	(b) 31 girls with a short stature and a recent pubertal onset	(b) Open trial	(b) FH prognosis: 1±2.3 cm greater than the pretreatment one	(b) Duration of treatment: 23±4 months	86
GnRHa + GH	(a) 12 girls with ISS and normal/early puberty	(a) Prospective controlled trial	(a) Mean height gain of 10 cm between pretreatment PAH and AH	(a) No adverse effects registered	91
	(b) 32 short adolescents	(b) Randomized controlled trial	(b) No differences in final height between the treatment and control groups; net height gain of 4.9 cm	(b) A tendency toward a lower mean lumbar spine bone mineral density	93
AI	31 ISS boys	Prospective, double-blind, randomized, placebo-controlled study	Increase of 5.9 cm in PAH	2 years of treatment No adverse effects on bone mineralization	99
AI + GH	76 ISS boys	Randomized 3-arm open-label study	Near FH gain of + 22.5 cm in subjects receiving GH + AI	Duration: 24–36 months No safety issue emerged	101
rhGH + rhIGF-I	106 subjects with a short stature, low IGF-I, and GH sufficiency	Randomized open-label trial	First-year HV of 11.2 ± 2.1 cm/year in the group treated with 45 µg/kg of rhGH and 150 µg/kg of rhIGF-I	Duration: 3 years Similar safety profiles between groups	104

Rh-IGF-I, recombinant human insulin-like growth factor-I; AH, adult height; FH, final height; PAH, predicted adult height; HV, height velocity.

treat children with a normal variant of growth destined to achieve a normal adult height was and is highly questionable [98], this study opened avenues of further therapeutic applications of AI in different conditions associated with short stature.

A prospective study conducted in 31 ISS boys treated with AI or placebo for 2 years showed an increased predicted adult height of 5.9 cm without adverse effects on bone mineralization [99]. A recent case report showed the effect of AI on the final height of a 14.5-year-old boy with ISS and a predicted adult height below –3 SD [100]. After a 5-year letrozole monotherapy, he reached a final height of –1.57 SD without apparent permanent side effects.

A randomized trial compared the effect of AI alone, rhGH alone, and the combination of AI + rhGH in 76 ISS pubertal boys treated for 24–36 months [101]. Among all the treatment regimens, the combined treatment (AI + rhGH) was the most effective therapy for improving growth with a good safety profile. The height gain in the course of treatment was +18.2 cm in subjects treated with AI, +20.6 cm in subjects treated with GH, and +22.5 cm in subjects receiving AI + GH.

Overall, AI therapy seems effective in improving predicted adult height [77], though the net effect on adult height remains uncertain and long-term data are limited. Safety issues emerged as an increased prevalence of vertebral anomalies was reported in boys with ISS who had started AI treatment before the onset of puberty [102]. Therefore, according to the available evidence, the use of AI should be considered experimental in growth disorders [77, 103].

Insulin-Like Growth Factor-I

Both GH and IGF-I stimulate growth, having complementary and synergistic actions. As their effects are additive, the combined treatment of rhGH plus rhIGF-I therapy may have a higher impact on growth than monotherapies.

A 3-year randomized open-label study was performed in short children with normal GH secretion and low IGF-I [104]. A significant increase in linear growth was observed only in subjects receiving the highest daily dose of the combined treatment (45 µg/kg of rhGH and 150 µg/kg of rhIGF-I). rhGH/rhIGF-I coadministration resulted

in a significantly greater height velocity at year 1 only, while still producing an overall greater height gain over the 3 years of the study. Although the safety profiles of the groups were generally similar, adverse effects such as headache and hypoglycemia were more frequent in the group receiving the highest doses of combined treatment.

Due to the lack of data on the impact of combination therapy on adult height, the lack of large-scale studies, the burden of rhGH/rhIGF-I coadministration on the patients and their families, and the associated high costs, this therapeutic approach is not recommended for children with ISS.

Final Remarks

Table 2 summarizes the main studies reporting the various therapeutic approaches in ISS subjects.

The available evidence clearly shows that there is no highly effective therapy for children with ISS. This is not surprising as ISS comprises a wide spectrum of disorders with different underlying pathophysiologies. Consistent with this, a broad individual variability in response to the various therapeutic approaches has been reported in all clinical trials published so far. The identification of responders remains the challenge and that, in other words, means the achievement of a correct diagnosis in order to drive the correct treatment if available or at least to avoid ineffective and upsetting treatments in nonresponders. Finally, no robust evidence exists about the potential negative impact of stature on psychosocial status and the quality of life of subjects with short stature with the exemption of the extremes of short and tall statures [105].

Conclusion

ISS is still a challenging and controversial diagnosis the definition of which should be revised and include stricter criteria ruling out subjects with CGDP. Indeed, ISS is an exclusion diagnosis which should be reserved for subjects without a recognizable cause of shortness. To date, most ISS subjects do not have a specific diagnosis but the improvement of diagnostic techniques will soon allow molecular characterization of a high proportion of ISS children, at least leading to a stratification of the ISS population. The identification of a particular defect would have important consequences as it may drive treatment decisions and favor a tailored management. Different therapeutic strategies have been tested in ISS subjects but, to date, no treatment has been effective in all children with ISS, due to the heterogeneity of conditions underlying ISS. Future studies performed in more selected populations are required to clarify the effectiveness of the different therapeutic options. Further research is needed to better categorize children with ISS according to both phenotype and genotype in order to obtain information that is helpful to drive a personalized approach.

Disclosure Statement

The authors declare that they have no conflict of interests to disclose.

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