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A Genetic Approach to the Evaluation of Short Stature of Undetermined Aetiology

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Abstract

Short stature remains one of the commonest presentations to paediatric endocrinologists. After excluding major endocrine or systemic disease the majority of children with short stature currently receive a diagnosis based on a description of their growth pattern and height of their parents, e.g. familial short stature. Height has long been identified as a polygenic trait and genome wide association studies have identified many of the associated genetic loci. Here we review the application of genetic studies including copy number variant analysis, targeted gene panels and whole exome sequencing in children with idiopathic short stature. We estimate 25 to 40% of children with a diagnosis of idiopathic short stature could receive a molecular diagnosis using these technologies. Achieving a molecular diagnosis is not only important for the affected individuals and their families but may also inform treatment decisions surrounding the use of GH or IGF-I therapy.

Search Strategy and Selection Criteria

We searched the Cochrane Library, MEDLINE, and EMBASE for articles published between 2000 and November 2016. We used the search terms idiopathic short stature, short stature or small stature combined with body height. We also combined human growth hormone with idiopathic short stature/short stature/small stature. Clinical trials, guidelines, meta-analyses, multi-centre studies, randomized controlled trials and systematic reviews were given preference. We also searched the reference lists for articles identified by this search strategies and selected those we judged relevant.

1. Introduction

Concerns about short stature are among the most common reasons parents seek consultation with a growth specialist, accounting for about half of new visits to a paediatric endocrine practice. Despite standard clinical and laboratory evaluation, a pathologic diagnosis is not reached in 50-90 % of cases. Most of these children are labelled as either constitutional delay of growth and puberty, familial short stature, or idiopathic short stature (ISS)¹⁻³, which are simply descriptive terms of overlapping populations. Although there is general consensus with regards to classification and effectiveness of therapies (e.g. a short course sex steroid for delayed puberty and recombinant human growth hormone [r-hGH] for ISS), there remains uncertainty and controversy about the diagnostic approach and treatment (r-hGH is not licenced for ISS in all countries), all occurring in the background of persistent ethical concerns about “medicalizing” variants of normal and the potential for harm by growth stimulating agents^{4,5}. Consensus guidelines address some of these issues, the most recent exclusively for idiopathic short stature were published in 2008⁶ and for the children born small for gestational age (SGA) in 2007⁷ and 2011⁸ with guidelines on GH treatment for short-statured children published in 2016⁹. Since then there have been significant changes in our ability to use molecular genetic tests to diagnose and classify these children.

The most widely accepted definition for idiopathic short stature (ISS) is a height more than 2 standard deviations below the mean for age and sex and where no aetiology is revealed following a detailed history, physical examination, and limited laboratory testing^{6,10}. Typically stature is below -2 SDs by age 2-3 years following a normal birth weight, and then either remains at this relative constant deficit or gradually falls to even lower percentiles during childhood with an acceleration of growth during puberty (FIGURE 1). Thus, ISS would include cases of familial short stature (a short child in the range expected given parental stature) and some cases of constitutional delay of growth and puberty, i.e. a child shorter than expected for parental size but with a delay in pubertal maturation.

One of the challenges for this review is how to deal with the arbitrary and perhaps misleading separation of ISS from other conditions. The postnatal growth pattern of the short child born SGA is similar to that of a child with ISS and the mechanisms responsible for the poor growth for most remain unknown^{6,11}. Furthermore many young patients with constitutional delay of growth and puberty are indistinguishable from those with ISS prior to puberty. Since all these are short at some time in life and the aetiology of the short stature unknown, they all could be considered to have “idiopathic short stature, i.e. ISS”. Thus, for this review we have chosen to broaden discussion and, in the absence of a specific diagnosis and will use the term short stature when referring to classical ISS and the related populations, including those born SGA.

Several reports have detailed spontaneous growth and adult height in children with ISS and generally reach the same conclusions¹²⁻¹⁴. Two thirds to three quarters achieve adult stature within the normal range although most are below average. As a group, mean adult height approximates -1.5 SDS. The increase in the height SDS as adults compared to that observed during childhood is due to a delay in pubertal onset of approximately one year, which extends the growing period. These observations are backed up by randomized controlled trials of r-hGH in ISS or short children born SGA, which show that untreated controls increase their height SDS score by the time they reach adult height¹⁵⁻¹⁸.

Most children with ISS reach adult heights in the normal range, yet it has proved very difficult to separate out who are at high risk for permanent short stature. Several factors are associated with poorer height outcome in the absence of treatment (e.g. shorter parents, lower predicted adult height based on stature/bone age)¹⁴. However, the influence of these factors is not robust enough to predict eventual height in an individual patient with sufficient accuracy.

Randomized controlled trials of r-hGH in ISS and short children born SGA demonstrate clearly that r-hGH accelerates growth and increases adult height when administered for several years. Increases in adult height SDS approximate 1 SD, but height gains vary among individuals and by study¹⁵⁻¹⁸. Treatment of short stature, with the exception of where the height deficit is sufficient to impede function or require adaptation, is undertaken with the expectation that a greater height will mitigate the adverse psychosocial effects of short stature and be of lasting benefit. Although some studies have attempted to examine such effects of r-hGH treatment, results have been variable and perhaps subjected to flaws in study design¹⁹. In the end it remains difficult to know whether for some reassurance and counselling may be a better treatment than daily injections. Presently the treating physician must weigh the pros and cons of intervention for each individual.

Although r-hGH is approved in several countries for treatment for short children with ISS without GH deficiency, the use of r-hGH in these conditions remains controversial because of the variable response to growth hormone therapy²⁰, uncertainty as to the exact benefit of the intervention, and concerns about long-term safety. Many of these issues stem from the fact that patients classified as ISS or related conditions are a mixture of pathologies that affect growth. As we now appreciate the wide variety of molecular genetic variants that impact stature, it is expected that these may be predictive of adult height, responsiveness to treatment, and risk of adverse effects. The purpose of this review is to provide an update on potential approaches to the evaluation of the child with short stature of undetermined aetiology, in particular focussing on genetic investigation and implications to management.

2. Current Diagnostic Approach

When no clear diagnosis for the short child is forthcoming from the history and examination, then a range of investigations can be undertaken, including testing to exclude occult system disease, surveying for subtle skeletal abnormalities, screening for hormone deficiencies and major genetic anomalies. Detailed recommendations on standard evaluation of short stature are provided in several publications^{6,21,22}.

Although a patient with ISS, by definition, is not GH deficient, there has been a great deal of investigation into the potential role of perturbations in the GH/IGF axis as a basis for their reduced stature. These studies were largely driven by the increased availability of r-hGH and data that defined the roles of GH and IGF as major regulators of skeletal growth^{23,24}. Because both GH secretion and responsiveness exist along a continuum (with severe GH deficiency and GH insensitivity residing at the ends of the spectrum)²⁵ subtle alterations in either GH secretion or action may underlie cases of ISS; this is supported by studies showing that circulating levels of IGF-1 are typically lower on average in ISS children^{26,27} with up to 50% of ISS children having subnormal levels^{26,28}. Therefore a short child with no clear diagnosis typically undergoes testing of the GH-IGF axis. The majority will have normal peak GH levels during stimulation testing with a normal or low IGF1 concentration. Treatment with r-hGH can be initiated in those countries that have licensed r-hGH for ISS or the short child born SGA; r-hIGF1 may be indicated for those with GH insensitivity/primary IGF deficiency⁹.

The skeletal dysplasias are a heterogeneous group of over 450 genetic disorders of cartilage and bone²⁹ and need to be excluded in patients classified as ISS. While these disorders often produce clinically obvious disproportionate short stature, there is wide phenotypic variability as evidenced by studies identifying short stature due to *SHOX*³⁰ or *FGFR3*³¹ mutations/deletions in patients with ISS without obvious dysplasia. Flechtner *et al*³² systematically evaluated the prevalence of skeletal dysplasias in a cohort of children referred to

an endocrine/growth disease centre. A skeletal dysplasia was identified radiographically in 51/234 (21.8%) ISS patients with an increase in prevalence where one parent was also affected (21/63, 33.3%, $p=0.009$). This high diagnostic yield may reflect ascertainment bias as the study was undertaken in a reference centre for skeletal dysplasias. None-the-less, the results underscore the need for considering skeletal dysplasia in patients with apparent ISS.

During the evaluation of a child for short stature disorders of imprinting must also be considered. The most widely recognised is Silver-Russell syndrome which is caused by maternal uniparental disomy of chromosome 7 (5-10% of cases) or hypomethylation of the paternally inherited allele at chromosome 11p15 (30-60% cases) which results in suppression of *IGF2* transcription³³. Recently a small number of families have been identified where Silver-Russell Syndrome has been caused by paternally inherited *IGF2* mutations³⁴. Short stature is also seen in Prader-Willi syndrome which results from paternal deletions at 15q11.2-q13, uniparental disomy of chromosome 15 or an imprinting defect³⁵. Pseudohypoparathyroidism is also associated with short stature and caused by a variety of genetic mechanisms including methylation abnormalities, maternal deletions or uniparental disomy of chromosome 20³⁶. Silver-Russell, Prader-Willi and pseudohypoparathyroidism are well described and familiar to most clinicians, Temple syndrome, caused by maternal uniparental disomy of chromosome 14, paternal deletions or imprinting defects at chromosome 14q32 is less well recognised³⁷. Clinical features of Temple syndrome include low birth weight, short stature, hypotonia, motor delay, early puberty, small hands/feet and obesity.

3. Rationale for more detailed genetic investigation

The role of specific genes as determinants of both normal and pathologic human growth has been actively investigated during the last decade, catalysed by tremendous advances in the ability to identify gene variants and their functional consequences. Early twin and adoption studies indicate that adult height is 80-90% heritable³⁸⁻⁴⁰. Thus far, genome wide association studies have explained 27.4% of the adult variation in human height with 780 variants identified from a population of 711,428 healthy individuals^{41,42}. This analysis includes effects of both common and rare variants on height. Of note the effect size of rare variants was more than 10 fold higher than that of the common variants. The current estimate that 27.4% of height can be explained by known variants may be an underestimate as analysis using imputed variants explains up to 56% of variation in height⁴³. Therefore it is near certain that the reduced stature of many ISS patients has a genetic basis. For some, this will be due to a variation in expression or function of a single gene product (monogenic) whereas for others the condition is the result of accumulation of multiple height impairing variants (oligogenic or polygenic). As well as height, age at menarche (and hence likely age at pubertal onset) also has a genetic basis having been linked to 397 genetic variants⁴⁴. Such genetic variants are likely to affect the tempo of growth and puberty and may contribute to the phenotype seen in CDGP.

4. Exemplars of targeted investigation

The single gene approach

These studies examine the frequency of mutations in candidate genes in ISS and related conditions. Perhaps the best example are studies of *SHOX* (short stature homeobox), the first genetic cause of ISS reported in 1997⁴⁵ and also a cause of Leri-Weill dyschondrosteosis⁴⁶. Mapping studies followed by sequencing identified a single *SHOX* mutation in 1/91 (1.1%) ISS patients, demonstrating that mutation of a single gene may cause syndromic forms of short stature in some children, while in others affecting stature with minimal additional clinical features. Subsequent studies now place the prevalence of *SHOX* abnormalities in ISS at between 3-15%⁴⁶⁻⁴⁹. In addition, genetic abnormalities within the pseudoautosomal region 1 (PAR1) of the sex chromosomes (where *SHOX* resides) have all been associated with short stature disorders and affect *SHOX* expression⁵⁰⁻⁵³. Interestingly, patients carrying a *SHOX* enhancer deletion are less disproportionate and respond better to GH therapy than patients with *SHOX* haploinsufficiency⁵⁴.

Mutation of *NPR2*, the gene encoding the receptor for C-type natriuretic peptide, represents another circumstance where the phenotype varies from clinically obvious skeletal dysplasia to ISS. Initially associated with the autosomal recessive skeletal dysplasia, acromesomelic dysplasia, the finding of reduced height in carriers in affected families⁵⁵ led to the identification of *NPR2* mutations in 6% of children with ISS⁵⁶ with 1/3

having disproportionate short stature. Another report identified 6 pathological *NPR2* mutations in a cohort of 95 ISS and 173 suspected Leri-Weill dyschondrosteosis patients (all with no known defect in *SHOX/PAR1*)⁵⁷.

Given the key role of the GH-IGF1 axis in growth and the common finding of low IGF1 in ISS patients^{26,27}, several studies have SOUGHT mutations in the genes encoding components of this axis in undiagnosed short patients. An increased frequency of mutations in such short children compared to controls has been found for the GH secretagogue receptor *GHSR*⁵⁸, *IGF1*^{59,60}, IGF acid labile subunit *IGFALS*⁶¹ and *IGF1R*³⁰. Recently loss of function mutations in pregnancy-associated plasma protein A2 (*PAPPA2*) have been identified in two families with previously unexplained short stature⁶². The patients presented with raised concentrations of GH, IGF-I, IGFBP-3, ALS and IGFBP5. *PAPPA2* encodes a metalloproteinase responsible for cleavage of IGFBP-3 and IGFBP-5, an essential step in the generation of free IGF-I. The prevalence of *PAPPA2* mutations in general population of short children has not yet been examined.

The multi-gene approach

The studies described above all focussed on a single or very limited number of genes, typically identifying the cause in 5 % or less of the population. The low yield is not surprising given the ~700 genes known to affect growth, but the studies serve as proof of principle that cryptic gene mutations are plausible causes of ISS. Targeted Next Generation Sequencing is more broad-based approach that has been used in cohorts of ISS subjects. Multi-gene studies typically analyse a host of genes that are either known to cause short stature, are linked to adult height by genome wide association studies or are known to be involved in growth through animal or *in vitro* studies. Two main approaches have been taken– a) assessing differences in SNP frequency between control and ISS populations in order to locate allelic variants influencing growth or, b) examining candidate genes for pathological mutations.

De Graaff et al investigated 225 SNPs in 10 genes linked to growth and glucose metabolism (*GHI*, *GHR*, *IGF1*, *IGF1R*, *STAT5A*, *STAT5B*, *MAPK1*, *MAPK3*, *PPARγ* and *INS*) in 1,437 ISS and SGA patients. Only one SNP (rs4966035, *IGF1R*) was significant and was associated with being SGA⁶³. In another study of 155 ISS patients and 318 normal controls, two SNPs within *HMG2A*, a gene where variants have previously been associated with adult height, were found to be associated with ISS⁶⁴. The EPIGROW study examined 232 candidate genes via next generation sequencing in 263 ISS and SGA patients. Two SNPs in *ZBTB38* 5'UTR and promotor region as well as two insertions/deletions (one in *IGF1* and one in *NFKB1*) were significantly different between cases and controls²⁸. Such studies indicate that gene variants contribute to short stature, but do not often provide a precise diagnosis. *HMG2A* (also called *HMGB3*) expression has been associated with poor prognosis for several tumour types⁶⁵⁻⁶⁷ while *ZBTB38* regulates DNA replication⁶⁸ and has been found to be mutated in a rare mucinous neoplasm and *NFKB1* insertion/deletions are also linked to cancer risk⁶⁹.

Wang et al⁷⁰ screened 1077 candidate genes (366 associated with known short stature disorders) in a heterogeneous cohort of 192 children with short stature (16% GHD, 15% developmental delay and 7% with a syndromic diagnosis but no identified mutation). A definitive diagnosis, where a known pathological variant was identified and the patient's phenotype matched that of the known variant, was made in only 4 patients (2%) – 3 cases of Noonan syndrome and 1 case of brachyolmia type 3. There were a further 64 variants identified from the 192 children where the variant was a previously identified pathogenic mutation but the patient phenotype did not match that previously reported. Some of these variants are likely to represent prior false attributions but some may also represent phenotypic variability and thus the diagnostic yield could well be higher than the reported 2%.

5. Genome-wide approach

These studies use less biased approaches to survey the genome for the putative cause of short stature. The methods employed can detect known pathologic variants in individuals who may manifest short stature as the only sign of a syndromic condition but also can lead to the discovery of novel genes and pathways involved in growth control. Most commonly these utilize either competitive genome hybridization (cGH) or SNP arrays to detect copy number variants (CNVs) or whole exome sequencing (WES) to examine coding regions of the entire genome. Usually CNV analysis is undertaken prior to WES as, unlike WES, CNV analysis is well established as part of routine clinical care and more widely available.

The human genome contains multiple regions of genomic DNA copy number gain or loss (i.e. CNVs) which can range from kilobase to megabase size⁷¹. cGH-arrays most often provide a genetic descriptor rather than a specific diagnosis. An array however can be an important step in the evaluation of ISS children, as it may suggest a more precise genetic diagnosis (e.g. deletion of a part of a chromosome that is the location of a key gene, e.g. the IGF1 receptor [*IGF1R*]). Copy number variant studies have revealed a range of abnormalities in short stature, as summarised below.

Dauber et al reported the relationship between stature and CNV frequency using in a cohort of 4411 individuals with childhood/early adulthood height data (mean height SDS -0.19)⁷². Subjects with a height SDS of <-2 SD had a 1.26 fold increase in average CNV length⁷²; thus there is an increased CNV burden in individuals with short stature. This increase in CNV burden was significant for deletions (but not duplications) with a population frequency of <5%, with rare deletions being increased 2.3 fold in the short individuals. This study, however, did not seek to identify individual pathogenic CNVs in the individuals with short stature but subsequent studies⁷³⁻⁷⁶ have attempted to address that question.

In a cohort of 200 individuals with short stature (131 isolated short stature and 69 syndromic short stature), Zahnleiter et al identified 20 patients (10%) with presumptive pathogenic CNVs, 10 were duplications and 10 deletions⁷⁵. Eight were associated with known microdeletion/duplication syndromes and a further two contained known stature-determinant genes. For the remaining CNVs there was strong evidence that they were pathogenic as all contained genes associated with adult height, growth pathways or a mouse growth retardation phenotype and in addition were either *de novo* or, where inherited, segregated with short stature in the family. Individuals with such CNVs had an increased rate of intellectual disability (9/20 CNV patients) and lower height SDS (-3.34 SD vs -2.75 SD) when compared to those individuals without a CNV. The probability of detecting a CNV was increased in subjects born SGA (15% SGA subjects vs 8% AGA subjects). In a similar study van Duyvenvoorde et al genotyped 149 families containing individuals classified as ISS or SGA and identified CNVs containing known short stature genes in 6 families (4%: 4 *SHOX* deletions/duplications and 2 *IGF1R* deletions)⁷⁶. In an additional 13 (8.7%) families a potentially pathogenic CNV was identified which was either *de novo* or segregated with short stature in the family. Additional studies report similar diagnostic yields⁷³. Future work to include functional studies is likely to refine the diagnostic yield from CNV studies by improving the classification of such CNVs as pathogenic and non-pathogenic. The use of SNP arrays is likely to improve diagnostic yield compared to array CGH as SNP arrays can also identify uniparental disomy.

Recently investigators have turned to WES for short stature disorders in order to uncover pathogenic alleles, both known and novel. Nilsson et al identified autosomal dominant aggrecan (*ACAN*) mutations in 3 families with ISS and an advanced bone age⁷⁷. The encoded protein, aggrecan, is an extracellular matrix proteoglycan, and *ACAN* mutations had previously been reported in patients with spondyloepimetaphyseal dysplasia⁷⁸. Guo et al examined 14 children with short stature (height SDS <-3) of unidentified cause using WES and reached a genetic diagnosis in 5 children (2 with 3-M syndrome and one each with Kenny-Caffey Syndrome, Floating-Harbour Syndrome and the progerioid variant of Ehlers Danlos Syndrome) giving a diagnostic yield of 36%⁷⁹. For two of these patients (Kenny-Caffey and Ehlers Danlos Syndrome), the phenotype was mild. The 14 patients recruited to the study by Guo et al were selected from a larger cohort of 192 patients and the diagnostic yield is likely to have been increased by the selection process and by the use of an inclusion criteria of height SDS <-3.

Thus far reports frequently assessed patients with dysmorphic features and/or radiological features of skeletal dysplasia and thus atypical for patients with ISS. However, it is likely that further application of exome sequencing in the SGA and ISS populations will identify additional patients with pathogenic mutations affecting genes associated with a syndromic diagnoses but who lack characteristic features.

6. Proposed Approach to the ISS child (figure 2)

We now suggest that for those ISS children with marked short stature (Height SDS <-3) undertaking more detailed genetic investigation should be considered to reach a precise diagnosis. There are many benefits to this. These include the removal of the need to undertake further evaluations - many of these children currently undergo batteries of expensive tests. Prognosis and counselling are much better informed when a diagnosis is reached, with screening for associated health issues. Genetic testing can also be undertaken in the wider family. There are, however, some drawbacks. A precise diagnosis removes the ISS label and in some

countries the licenced indication for r-hGH. Conversely, identification of a *SHOX* mutation could provide a licensed indication for r-hGH when treatment of ISS is not approvable. A novel variant may be identified, which is predicted to be pathogenic, but cannot be confirmed as causative without other reports and/or complex functional analysis. In our opinion, benefits outweigh drawbacks and our suggested approach illustrated in Figure 2. The algorithm describes scenarios that either suggest particular lines of investigation depending on the presence or absence of clinical features.

Patients with subtle features suggestive of a particular condition (e.g. Scenario 2, *SHOX*, *NPR2* or *ACAN* deficiency) should be prioritized for individual gene sequencing. A higher prevalence of *SHOX*/*PAR1* abnormalities is seen among ISS patients where there are some features of Leri-Weill dyschondrosteosis such as disproportionate short stature^{48,80} or a family member with Madelung deformity⁴⁷. There will remain, however, a considerable number of patients with pathogenic *SHOX* variants who have body proportions within the normal range, this is especially common in those patients with deletions of *SHOX* enhancers and without increased routine genetic analysis these patients will not receive appropriate therapy. In addition, the prevalence of *NPR2* mutations in an ISS population depends on the extent of anthropometric and radiographic assessments⁵⁷. Therefore careful measurements of body proportions (e.g. sitting height, arm span, upper-lower segment ratio, and head circumference) and a skeletal survey would identify those ISS children on whom a targeted search for mutations in these genes would be worthwhile. Techniques and reference ranges for these measures in North American can be found at <https://www.cdc.gov/nchs/nhanes/index.htm>. Norms for other populations are also available^{81,82}.

In the absence of phenotypic or biochemical pointers, one route to increase molecular diagnosis rates in children would be the use of either targeted panels of genes linked to growth disorders or high resolution chromosomal microarray analysis (e.g. scenarios 3 and 4). CGH or SNP arrays detect pathogenic CNVs in 17-19% of patients with developmental delay or intellectual disability, a finding which prompted the American College of Medical Genetics and Genomics to recommend chromosomal microarray analysis as a first tier clinical diagnostic test for these individuals⁸³. For ISS children, a diagnostic yield of 5-10% for identifying CNVs known to be associated with short stature is expected based on published studies. Another 5-10% of patients have CNVs which are highly likely to be pathogenic, giving a potential total diagnostic yield of 10-20%⁷³⁻⁷⁵.

With the falling cost of next generation sequencing (NGS, exome and whole genome), this approach is likely to play an increasingly useful role in the evaluation of children with unexplained short stature. The threshold such testing is arbitrary and dependent on the presence of clinical features that raise the chance of detecting of an important genetic anomaly. The review by Dauber et al⁸⁴ outlines an approach for diagnosis of suspected monogenic growth disorders. Diagnostic yield will be highest in patients with severe short stature, additional affected family members (while both affected and unaffected family members will increase yield from exome sequencing the presence of two or more affected family members raises the probability that the cause of short stature is likely to be monogenic in origin), and dysmorphic features (i.e. children in whom there are dysmorphic features but in whom a clinical syndromic diagnosis has not been made). In short children without affected family members, normal biochemistry and no dysmorphic features the diagnostic yield is likely to be low but over time will improve as further genetic causes of short stature are identified. Interpretation of whole exome/genome data requires intensive bioinformatics support and clinical experience to correctly interpret the results. Further studies evaluating larger cohorts of unselected ISS and SGA children are required to evaluate the diagnostic yield and current cost-effectiveness of using NGS sequencing in these cohorts. Using copy number variant analysis, targeted single gene sequencing and next generation sequencing, we estimate that up to 25-40% of the children currently diagnosed with ISS and height SDS <-3 could receive a more specific diagnosis. As our knowledge of the genetic causes of ISS increases, the diagnostic yield from whole exome/genome studies is likely to increase, which in combination with the rapidly falling costs of sequencing is likely to lead to the routine clinical use of this technology in short stature patients.

7. Therapeutic implications

It remains a challenge to select the ISS patients and those with related conditions most likely to benefit from the use of growth-promoting agents. Treatment is generally more effective when initiated at a young age, but that is a time when it is very difficult to predict adult height for those left to follow their natural course.

Achieving a specific diagnosis therefore has the potential to aid in treatment decisions. For example, the finding of an *IGFALS* defect in a child with moderate growth impairment could lead to a decision not to treat at all, as many patients naturally achieve a normal adult height and rhGH treatment may not be efficacious⁸⁵⁻⁸⁷. Additionally in the SGA population, NGS may reveal a diagnosis such as Fanconi or Bloom syndrome, in which growth-promoting treatments would be contraindicated because of risk for malignancy. The finding of an *IGF1R* mutation would support the use of r-hGH to attempt to overcome this receptor block^{88,89}. Reported evidence of the effect of r-hGH in those with *ACAN* mutations could support a trial of treatment⁹⁰.

There is also the potential for genetic information to guide dosing decisions or to determine whether r-hGH or r-hIGF1 is the preferred treatment. Up to 50% of ISS children have a low IGF-I level in association with normal or even high GH secretion²⁸. This may indicate a degree of GH insensitivity, requiring either higher r-hGH doses or treatment with r-hIGF1. A common isoform of the GH receptor (exon 3 deletion) has reduced GH signalling *in vitro* and thus appeared to be a good candidate for predicting GH sensitivity *in vivo*⁹¹. Although most studies show that harbouring the variant has limited impact on the growth in response to r-hGH⁹², the potential for other genetic variants in ISS to affect responsiveness remains high given the varied outcomes in ultimate stature for r-hGH-treated ISS patients and the convincing evidence that they display a range of sensitivities to GH and IGF1⁹³.

Some children will meet criteria for initiating rh-IGF1 treatment. This 'biochemical' diagnosis of primary IGF deficiency is classically associated with mutations in the GH-IGF pathway including the GH receptor, *STAT5B*, *IGF1* and *IGFALS*, but can also be seen in the rasopathies, defects associated with the NF-kB pathway⁹⁴ and various syndromes e.g. 3-M⁹⁵. Therefore the identification of an abnormality in the *GHR*, *STAT5B* or *IGF1* genes in an ISS child would mean that r-hIGF1 treatment could be considered, in particular if r-hGH has been tried and failed. In other circumstances where IGF1 bioavailability is adversely affected, as found the newly described *PAPPA2* deficiency⁶², r-hIGF1 may have a therapeutic role⁹⁶.

8. Conclusions

Decades ago evaluation of growth disorders was largely restricted to auxological description and treatment employed limited amounts of pituitary-derived growth hormone in select individuals. The advent of radioimmunoassay led to the ability to measure hormones in the bloodstream followed by the elucidation of growth hormone signalling mechanisms involving the IGFs, receptors, etc. Subsequently the field advanced to where a patient's condition was defined by hormonal profiles and the status of their growth hormone/IGF axis. The focus on growth hormone and its partners was logical given the pronounced role in growth regulation (GH and IGFs being responsible for about 70% of growth of mammals⁹⁷) and the availability of r-hGH and later r-hIGF1 as therapeutic agents.

Our perspective on the determinants of stature has broadened tremendously over the last decade, driven by ever more sophisticated and extensive studies of the genome (Figure 3). Many of the genes encoding proteins required for normal growth in height are expressed in the skeletal growth plate, from which all linear growth is derived⁹⁸. Thus, we now enter a time when genetic data will be used to better define and diagnose growth disorders, expanding our knowledge much as hormone measures did in the past. Such testing should augment the evaluation, not replace auxological data and hormone measures. Assessment of the GH/IGF axis remains key as many presently available interventions involve this pathway. With technological advancements allowing the assay of genetic information proceeding extremely rapidly, genetic tests certainly will soon become common place in the routine evaluation of growth disorders and useful to reach specific diagnoses as well as guide treatment.

Given the power of currently available genetic analyses and high heritability of height, it is perhaps somewhat disappointing that diagnostic yields are not higher, especially in non-syndromic ISS. However, the complexities of genetic expression/interaction far exceed what most of us imagined. The current approach is predominantly Mendelian, using tests that seek to explain the growth deficit by a defect in a single gene. The limitation of this is evidenced by the fact that polymorphisms at 700 loci explain only half of heritability of stature in humans. While ISS will be the result of a single gene mutation having a profound impact on growth in some patients, for others their growth attenuation will be the result of multiple gene variants, each variant having a lesser effect but collectively leading to significant growth impairment. In addition, changes in the expression of growth-determining genes resulting from epigenetic mechanisms such as cytosine methylation,

390 histone acetylation, or microRNA abundance remain to be explored. Though our inability to fully examine
391 these mechanisms stands as a limitation at present, it also offers an opportunity to expand our understanding of
392 growth control and eventually explain the basis of short stature in the majority of those affected.
393

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651 TABLE

652 Examples of Known Monogenic Causes of Growth Disorders

Mechanistic Class	Representative Monogenic Causes	Typical Phenotype
Insulin/IGF Pathways	<i>Insulin-like Growth Factor 1 Receptor (IGF1R) Insulin-like Growth Factor 1 (IGF1), Insulin-like Growth Factor 2 (IGF2), Insulin Receptor (INSR),</i>	<i>SGA and postnatal growth retardation. Dysglycemia Common.</i>
Growth plate and Skeletal Dysplasia	<i>Short Stature Homeobox (SHOX), Naturetic Peptide Receptor 2 (NPR2), AggreCAN (ACAN), Fibroblast Growth Factor Receptor 3 (FGFR3)</i>	<i>Variable features of skeletal dysplasia, some present as typical ISS</i>
DNA replication/repair, chromosome structure and transcription	<i>Chromatin Helicase DNA binding Protein 7 (CHD7), Fanconi Anaemia Complementation Groups (FANCA, FANCC, FANCG), Cell Division Cycle 6 (CDC6), Centromeric Protein J (CENPJ)</i>	<i>Increased risk of malignancy, SGA with microcephaly</i>
Signal transduction, Rasopathies	<i>Protein-Tyrosine Phosphatase Nonreceptor-type 11 (PTPN11), V-Raf-1 Murine Leukemia Viral Oncogene Homolog 1 (RAF1), Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS), Phosphatidylinositol 3-kinase Regulatory Subunit 1 (PIK3R1),</i>	<i>Normal size at birth, normal head size, postnatal growth impairment, dysmorphism.</i>
Ubiquitination and Protein Folding	<i>Cullin 7 (CUL7), Obscurin-like 1 (OBSL1), Coiled Coil Domain Containing 8 (CCDC8), Tripartite Motif-Containing Protein 37 (TRIM37), Ubiquitin Protein Ligase E3 Component N-Recognin 1 (UBR1),</i>	<i>SGA with normocephaly and normal intelligence.</i>

653

654 **Legend** Conditions have been divided by the mechanism underlying the growth impairment and a broad phenotypic description given for each mechanistic group. A
655 more extensive listing of monogenic short stature disorders can be found in the review by Wit et al⁹⁹

656

657

11. Figure Legends

Figure 1. Typical growth Curves for: A) average male, B) Constitutional Delay of Growth and Puberty, and C) Idiopathic Short Stature (ISS). Arrow indicate age of peak pubertal growth velocity.

Figure 2. Proposed schema for molecular diagnosis in the child with *Short Stature of Undetermined Aetiology*. The latter is defined as short stature <-2 SDS, $\pm$ being born small for gestational age, with no readily recognisable syndrome diagnosis, no significant microcephaly, and screening investigations normal (e.g. karyotype [in females], routine bloods to exclude occult system disease, thyroid function) and not GH deficient by standard GH stimulation testing. Each box contains a potential clinical scenario leading to an investigation strategy.

Clinical Scenario 1: The short child or the short adolescent with delayed puberty with no clinical features of system disease, endocrinopathy or skeletal disorder, and in some a family history of a similar growth pattern.

Clinical Scenario 2: The short child/adolescent with features that could indicate the presence of a skeletal disorder (minor disproportion, any bone length/size abnormality, advanced bone age).

Clinical Scenario 3: A child with more significant short stature (Height SDS <-3) with minor dysmorphic features suggestive but not in itself diagnostic of a known syndrome.

Clinical Scenario 4: GH-IGF testing could indicate a defect in the axis e.g. high GH levels and low IGF-I [*GH receptor* mutation] / normal GH and high IGF-I [*IGF-I receptor* mutation] / very high IGF-I and IGFBP-3 levels [*PAPP-A2* mutation] / undetectable acid labile subunit levels [*ALS* mutation].

Clinical Scenario 5: A child with more significant short stature (Height SDS <-3) with minor dysmorphic features not suggesting a diagnosis $\pm$ a family history.

Figure 3. Advancement in genetic approach to short stature by decade. Turner syndrome was first noted to be due monosomy X and later expanded to include other abnormalities of sex chromosome complement (mosaicism, partial deletions). Four decades later, *SHOX* deficiency was found to be the cause of Leri-Weill syndrome and also in patients with non-syndromic short stature. *SHOX* deficiency also contributes to the short stature in Turner syndrome. Silver Russell syndrome (a condition with both prenatal and postnatal growth retardation) is due to epigenetic changes in methylation that restrain IGF-2 expression. Genome wide scans for copy number variations (CNV) and application of Next Generation Sequencing (NGS) are being currently utilized with increasing frequency. Application of whole genome sequencing (WES) and more complex analyses are anticipated for the future.

Figures:

Figure 1

Figure 2

Figure 3