Genetic Techniques in the Evaluation of Short Stature

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KEYWORDS
- Short stature
- Height
- Genome-wide analysis
- Microarray analysis
- Whole-exome sequencing

KEY POINTS
- Many children with short stature do not have a cause to explain the disorder.
- Several genetic techniques, some of which are clinically available and others at the research stage, are helping to identify potential variants to explain short stature.
- Understanding these techniques will assist clinicians in their appropriate use and also help to foster support in expanding genetic screening to delineate mechanisms of poor growth.

INTRODUCTION

Normal growth is a complex dynamic process dependent on the coordination of multiple factors, including genetics and nutrition, as well as biological factors such as hormones, all working in balance. Any acute or chronic pathologic process may interfere with normal growth.\textsuperscript{1} The typical endocrine evaluation comprises a history, physical examination, and laboratory testing, including hormone levels and radiological studies. A genetic evaluation usually depends on initial clinical and laboratory findings. In many cases an endocrine, nutritional, or chromosomal abnormality is not apparent. However, the expansion of genetic technology has increased the diagnostic yield of genetic testing. Ongoing research efforts to identify genes influencing growth will provide a better understanding of mechanisms underlying abnormal growth and will eventually lead to novel management approaches.

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This article focuses on describing some of the current advances in genetic testing that can be used as part of the diagnostic evaluation of children with short stature. This article is not intended to be comprehensive, but rather to educate the reader on the increasing efficiency of genetic screening tools that will eventually provide clinicians with important diagnostic capabilities for the evaluation of children with abnormal stature.

GENETIC TESTING STRATEGIES

The approach to understanding disorders at the level of the DNA currently has several options. Depending on the clinical phenotype or the specific trait of interest, clinicians or investigators have several choices for how to screen a patient or cohort of patients. Some testing is commercially available, whereas other genetic tests have not been fully validated or standardized and are only available through research protocols. This testing ranges from conventional chromosome analysis and microarray analysis, to sequencing of single genes, sequencing of a panel of genes, or to whole-exome sequencing (WES). Each technique has advantages as well as limitations. The article explores how these techniques have affected clinical practice and helped further the understanding of the mechanisms of growth. Abnormal expression of imprinted genes has been associated with overgrowth and poor growth in syndromes such as Beckwith-Wiedemann and Russell-Silver syndrome. Methylation testing to detect active and inactive genes in imprinted areas of the genetic material is also available to screen children presenting with features suspicious for these syndromes. However, this group of disorders is not discussed in detail.

CHROMOSOME ANALYSIS

Chromosomal analysis or karyotyping has become an effective and efficient tool in the diagnosis of patients with short stature. It involves the visual examination of G-banded chromosomes in order to detect large structural deletions and duplications, monosomies, trisomies, and balanced rearrangements. Typically, these patients present with characteristic phenotypic features that include short stature. An example is mosaic Turner syndrome; however, short stature in a female patient may be sufficient justification to study chromosomes and thus has been argued as means to identify Turner syndrome at an earlier age. In the case of tall stature, phenotypic features of Klinefelter syndrome (XXY), is another example in which the evaluation of chromosomes is useful. Fluorescence in situ hybridization (FISH) can complement karyotyping in order to detect various small chromosomal abnormalities, such as deletions and duplications. This technique has been used, for example, to detect whole-gene deletions of the short stature homeobox-containing gene (SHOX), which have been associated with Leri-Weill dyschondrosteosis (LWD), but have also been implicated as a cause of short stature in children in whom full endocrine evaluations have not identified causes for their poor growth.

A karyotype with FISH may also detect specific suspected microdeletion syndromes. Deletions of chromosome 22q11.2 are among the commonest of all microdeletions and are associated with short stature, often in conjunction with other birth defects, typically congenital heart defects or cleft palate (DiGeorge or velocardiofacial syndromes). However, the presence of a supernumerary chromosome derived from chromosome 22 has been reported to cause cat-eye syndrome. It is associated with anal atresia, coloboma of the iris, ear malformations, and short stature, and in rare cases growth hormone (GH) deficiency. In another example, there is a...
description of a patient with an Xp13.2 chromosome microduplication, who presented with features similar to Turner syndrome that included short stature.6

Karyotyping and FISH are easily accessible to clinicians and economical, but have been low yield in cases of short stature without a cause. Most children with chromosomal anomalies and microdeletion/duplications have dysmorphic features, congenital anomalies, and intellectual disability, and treatment of short stature would be of limited utility. Nevertheless, the recognition of phenotypic features would be important in determining priorities for care and would alert clinicians to the risk of associated comorbidities. In isolated short stature, a chromosomal cause is unlikely. For example, identifiable gene deletions in the SHOX gene for patients who present with short stature reportedly only yields a diagnosis in 2% to 15% of this patient population.7

TARGETED OR MULTIGENE SEQUENCING

Phenotypic or hormonal abnormalities may prompt the investigation of a specific gene abnormality. For example, a suspicion of a skeletal dysplasia because of disproportionate short stature may prompt testing of the FGFR3 gene. Suspicion of a rasopathy such as Noonan syndrome can prompt specific testing for PTPN11 and related genes in the ras-MAPK pathway. Commercial laboratories now offer multigene panels for testing of proportionate and disproportionate short stature. This approach has led to identification of mutations in genes that may not be suspected on the clinical evaluation. The biggest limitation of using multigene panel testing and newer next-generation sequencing (NGS) technology to screen large numbers of genes is the identification of sequence variants that are difficult to interpret (variants of unknown significance).

In patients with short stature, much focus is placed on identifying abnormalities within the hypothalamic-pituitary-GH axis. These studies typically use a targeted gene approach, which may include screening for mutations in GHT, GHRH receptor (GHRHR) or insulinlike growth factor 1 receptor (IGF-1R). Although researchers have been able to identify genetic perturbations leading to abnormalities in hormone secretion, hormone viability, or receptor sensitivity, those patients who have identified genetic causes in this axis continue to be rare. Even in those patients with identified genetic abnormalities, there is often no clear genotype-phenotype correlation among patients with similar mutations.8 Therefore clinical testing for specific genes involved in the GH axis has limited utility at this time.9,10 A recent report characterizing the lack of differences in the auxologic and biochemical parameters of short children with SHOX variants compared with children with normal SHOX also helps to make this point.11

GROWTH HORMONE DEFICIENCY

Isolated GH deficiency (IGHD) is a rare diagnosis with several described mutations in both the GH1 and the GHRHR gene, but no cases of GHRH gene mutation have been recorded. Less than 15% of patients with IGHD have an identified gene defect, although the rate increases up to almost 35% with investigations of familial cases.12 A recent report describes an autosomal form of IGHD over 3 generations secondary to a heterozygous missense mutation in the GH-1 gene leading to misfolding of the protein.13 This type of IGHD is known as type II GH deficiency (GHD) and the severity of the phenotype has been correlated with the type of mutation identified in patients.14,15 In these cases in which GHD is thought to be secondary to mutations affecting splicing of GH1, there is production of deleterious isoforms leading to a dominant negative effect on the secretion of the 22-kDa full-length peptide.15,16 The
height deficit in these patients is variable and there is also a risk for developing additional pituitary hormone deficiencies.

Recently, a heterozygous mutation in the POU1F1 gene (p.Pro76Leu), which maps to a conserved site within the transactivation domain, was described as a cause of IGHD. Classically, mutations in the POU1F1 gene lead to a clinical phenotype triad of somatotroph, lactotroph, and thyrotroph dysfunction. Functional studies of this mutation show how this mutation can alter specific transactivating functions as well as interactions with specific cofactors such as ELK1, which activates endogenous GH.17 These studies report not only a novel mutation in POU1F1 leading to IGHD but further elucidate the complexities of pituitary development and function.

MULTIPLE PITUITARY HORMONE DEFICIENCIES

Along the spectrum of defects along the GHRH-GH axis, results of ongoing research continue to help clinicians better understand the development of multiple pituitary hormone deficiencies (MPHDs), of which abnormal stature is typically a part. Nevertheless, this field continues to be complicated because clinical phenotypes are typically not well predicted by genotypes.18,19 MPHD is often a dynamic progression of disorder because patients may develop additional hormone deficiencies over time. Although it is clear that idiopathic MPHD results from aberrations in pituitary development that may affect structure, cellular function, or even cell survival, most of these disorders do not have an identifying mutated gene and may suggest that MPHD is most likely not a single-gene abnormality. Despite this idea, several mutated genes have been reported as likely causes of the development of hypothalamic and pituitary disease.8,20,21 A better understanding of pituitary development and its complex regulation will most likely help to provide insight into potential pathologic mechanisms leading to hormone dysfunction.22 There is still much unknown about the coordination of the events, including expression of transcription factors and signaling genes, required for a process that is temporally and spatially dependent in order to produce a functional gland. Table 1 summarizes several of the reported genes shown to cause short stature and, in most cases, other endocrine abnormalities.

TARGETING BONE GROWTH

In bone growth, both C-type natriuretic peptide (CNP) and its receptor, natriuretic peptide receptor-B (NPR-B), have been cited as important regulators of endochondral bone growth.23 Studies using mouse models in which there is a loss-of-function mutation of the Npr2 gene or loss of CNP show that these conditions result in disproportionate dwarfism and abnormal endochondral ossification.24,25 In addition, disruption of the CNP-mediated signaling via cGMP in both in vivo and in vitro studies leads to skeletal defects at the growth plate.26 The importance of these factors in humans has also been recognized in studies of normal and abnormal growth.27,28

Homozygous mutations in NPR2 are associated with a severe dysplasia known as acromesomelic dysplasia, type Maroteaux (AMDM; OMIM [Online Mendelian Inheritance in Man] #602875).29 These patients have severe short stature, shortening of extremities, and bowing of the forearm, which clinically is similar to LWD (OMIM #127300). Despite this extreme phenotype with homozygous mutations on this gene, some investigators have described heterozygous mutations of NPR2 as a potential cause of disorder in patients otherwise classified as ISS.30 In one small study of 47 patients diagnosed with ISS, the investigators reported that 6% of the patients had heterozygous NPR2 mutations.30 In a larger group of patients with short stature, only 2% of the patients had identified heterozygous NPR2 mutations.31 Given the
clinical similarities of AMDM and LWD and that in cases of LWD about 30% of patients do not have identified SHOX mutations, other investigators have screened patients with LWD for mutation in NPR2. Only 3% of patients in a pool of 173 had NPR2 mutations, which included a total of 7 variants; however, functional studies confirmed that 6 of the NPR2 variants were pathogenic mutations.32

Despite these low percentages, it needs to be considered that these studies looked at small numbers of patients and, in addition, the phenotypic variability seen in short stature may account for a seemingly limited role of NPR2. An earlier study predicted that up to 1 in 30 individuals with idiopathic short stature (ISS) are carriers of NPR2 mutations.33 More recently, another study of patients with both short stature and normal stature provided further evidence of the impact of heterozygous mutations in NPR2 and concluded that patients with ISS should be screened.34 The investigators reported that NPR2 loss-of-function variants may account for 0.4% to 4% of patients with short stature and is associated with mild and variable growth impairment without a distinct skeletal

Table 1

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Subtypes</th>
<th>Gene Mutation</th>
<th>Description</th>
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<tr>
<td>IGHD 1A</td>
<td>GH (gross deletion, microdeletion)</td>
<td>Absent GH, severe short stature, development of anti-GH Abs after treatment with GH, tx with IGF-1</td>
<td></td>
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<tr>
<td>IGHD 1B</td>
<td>GH (splice-site mutations)</td>
<td>Short stature, tx with rGH</td>
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<tr>
<td>IGHD II</td>
<td>GH (splice-site mutations, intron/exon splice enhancer, missense mutations)</td>
<td>Short stature, tx with rGH</td>
<td></td>
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<td>IGHD III</td>
<td>—</td>
<td>HESX1</td>
<td>Associated with septo-optic dysplasia or MPHD. Deficiencies: GH, TSH, gonadotropins, evolving ACTH</td>
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<tr>
<td>Multiple pituitary hormone deficiency</td>
<td>—</td>
<td>PROP1</td>
<td>Deficiencies: GH, TSH, Prl, possibly gonadotropins, ACTH; associated hypoplastic or enlarge pituitary</td>
</tr>
<tr>
<td>—</td>
<td>POU1F1 (PIT1)</td>
<td>Deficiencies: GH, TSH, Prl; hypoplastic pituitary</td>
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<td>—</td>
<td>LHX3</td>
<td>Deficiencies: GH, PRL, LH, FSH, and TSH; associated with rigid cervical spine/limited head rotation</td>
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<td>—</td>
<td>LHX4</td>
<td>Deficiencies: GH, variable TSH, LH, FSH, ACTH; possible a target of POU1F1</td>
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<td>OTX2</td>
<td>Deficiencies: GH, TSH, LH, FSH, ACTH; associated with eye abnormalities</td>
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<td>—</td>
<td>SOX2</td>
<td>Deficiencies: LH, FSH, variable GHD; anophthalmia/microphthalmia, hypothalamic harmartoma</td>
<td></td>
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<td>—</td>
<td>GLI2</td>
<td>Deficiencies: GH, TSH, LH, FSH, ACTH; holoprosencephaly, craniofacial abnormalities</td>
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Abbreviations: Abs, antibodies; ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; Prl, prolatin; rGH, recombinant growth hormone; TSH, thyroid-stimulating hormone; tx, treatment.
phenotype. These conclusions are further supported by the finding that, when familial cases were analyzed, 13.6% of cases had \textit{NPR2} loss-of-function variants. In contrast with these studies, the importance of CNP in the regulation of human endochondral bone growth has also been suggested in studies of patients with bone overgrowth.\textsuperscript{35} Further support for the importance of \textit{NPR2} is shown in a report of a family with tall stature and macrodactyly of both great toes; all were found to harbor a gain-of-function type mutation in the \textit{NPR2} gene.\textsuperscript{36}

**GENOMIC TECHNOLOGIES**

The ability to scan the human genome has provided science with powerful tools to identify novel genes and study their role in normal physiology. Because normal variation in height is not only a heritable trait but also a polygenic trait, the advantages of genome-wide association studies (GWAS) are clear.\textsuperscript{37-39} It has been reported that GWAS have been able to identify hundreds of variants associated with human traits, but may not necessarily account for phenotypic variation. An earlier report using a genome-wide scan reported 20 variants associated with adult height, although these accounted for only approximately 3% of the height variation in the studied population.\textsuperscript{40} Genome-wide analysis studies have also helped to identify at least 180 loci statistically associated with genes that underlie skeletal growth defects.\textsuperscript{41}

Given the vast amount of information provided by analyzing the whole genome, strategies can be designed to only study a more select group of genes, such as in a microarray, or conduct more global screening, such as is seen with WES or even whole-genome sequencing. Each of these strategies provides advantages as well as disadvantages, but nevertheless has continued to improve understanding of the complexities of stature.

**CHROMOSOME MICROARRAY ANALYSIS**

Chromosome microarray or array Comparative genomic hybridization (CGH) is an approach that uses hybridization of a patient’s DNA and reference DNA to known DNA sequences, which can be oligonucleotides or single nucleotide polymorphism (SNP), from across the genome, in order to detect submicroscopic deletions and duplications. This technology can also detect uniparental disomy by detecting areas of SNP homozygosity. Copy number variant (CNV) is a term that refers to either heritable or novel variations in the number of gene copies. These duplications or deletions, which can include 1 or several genes, can be benign genetic variations in humans or a cause of pathologic phenotypes.\textsuperscript{42} This condition has been investigated as mechanism of disorder in other phenotypes with variable yield.\textsuperscript{43,44}

Some groups have reported CNVs in up to 10% of populations with short stature and height 2 standard deviation (SD) scores less than the average with no underlying cause to explain stature.\textsuperscript{45,46} Although the traditional candidate gene approach has allowed clinicians to diagnose monogenic defects leading to short stature, some believe that studying patients with extremes in stature (short or tall) in order to identify CNVs could potentially identify CNVs that include novel genes leading to short stature.\textsuperscript{46}

In one study of 162 patients with short stature, the investigators identified 40 potentially pathogenic CNVs in 33 families; however, further investigation and segregation analysis of these identified genes, as well as the previous documented literature, led to the conclusion that only 4% of these CNVs were confidently associated with short stature.\textsuperscript{46} One recent report found that CNV analysis in a family with short
stature revealed that the affected patient carried a paternally inherited heterozygous IGF-1 gene deletion, thus suggesting that CNVs may play a role in disrupting the GH–IGF-1 axis.47 Despite seemingly low yield in identifying target genes, earlier studies suggest that patients with short stature carry a greater global burden of CNVs. Nevertheless, these seem to be lower frequency CNVs and in the context of a polygenic trait such as stature, how much CNVs contribute to normal physiology versus disorder continues to be under investigation.48

Array-based comparative genomic hybridization/chromosome microarray analysis has been used in the study of children born small for gestational age (SGA) who have failure of catch-up growth. Some clinicians have used a candidate gene approach (SHOX, IGF-1, and IGF-1R) to identify a genetic cause, but this is reported to account for approximately 6% of these short children.49 However, array-based genomic copy number analysis found that 16% of this population may carry rare pathogenic or probably pathogenic CNVs.50 In another study, 27% of SGA patients studied with whole-genome SNP array analysis had identified CNVs that could potentially explain the short stature.51 The CNVs that were considered potentially pathogenic included several potential candidate genes previously implicated in GWAS studies of height or skeletal disorders in rodents.41,51

Because prenatal and postnatal growth in SGA children is polygenic and influenced by maternal, placental, and fetal factors, this could be considered as a cohort of patients who could provide better insight into understanding genetic determinants of growth and development. However, some clinicians think that gene variants only account for a small fraction of genetic variation and genome scans may be more helpful for focusing on rarer variants to gather insight into the significant pathologic mechanisms.52

WHOLE-GENOME AND WHOLE-EXOME SEQUENCING

The technology referred to as NGS, which is also known as high-throughput sequencing, has made it possible to screen large numbers of genes rapidly. It can provide higher efficiency in identifying pathologic genes and is less labor intensive than selective gene analysis. Furthermore, given the limitations of current diagnostic modalities in endocrinology and conditions in which disorders may evolve over time, NGS is a possible tool to identify novel genes. The limitation of this technology is in interpretation of sequence variants detected by analysis. Multiple tools are used to help interpretation, including population frequencies, disease frequencies, in silico protein prediction, family segregation, and algorithms that have been devised to standardize its interpretation. This technology would be more useful for identifying rare variants versus more common variants.52

One study screened patients with short stature for more than 1000 genes, many of which have been associated with growth disorders, skeletal dysplasia, growth plate biology, or GH signaling, and reported 1349 novel variants. The investigators highlighted probably pathogenic variants in IGF-1R and known pathogenic variants in the PTPN11 gene, which is associated with Noonan syndrome.53 In a larger study, the investigators reported almost 700 variants in 423 loci in tissue types and pathways involved in growth.54 These variants were reported to account for 36% of the heritability of adult height.

An important application of NGS is in the development of WES, which is a high-throughput genetic analysis in which the coding DNA of patients is sequenced. Although the exome is about 1% of the entire genome, it provides a more efficient method of analysis and limits the massive amounts of data screened. It has been
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<tr>
<th>Technique</th>
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<th>Indications</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Other</th>
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<tr>
<td>Chromosome analysis</td>
<td>Karyotype Microscopic G-banded chromosomes</td>
<td>Short stature associated with syndromic features, multiple anomalies,</td>
<td>Economical</td>
<td>Limited to syndromic short stature with low yield because of limited resolution</td>
<td>Used in combination with other cytogenetic techniques such as FISH (eg, diagnosis of SHOX haploinsufficiency)</td>
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<td>Microarray analysis</td>
<td>Comparative hybridization of patient and reference</td>
<td>Similar to chromosome analysis</td>
<td>Efficient screen for copy number variation. Can detect uniparental disomy.</td>
<td>Detects variants of unknown significance that may be difficult to interpret</td>
<td>—</td>
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<tr>
<td>Molecular diagnostics</td>
<td>Single-gene analysis</td>
<td>Targeted screening of specific genes using Sanger sequencing</td>
<td>Detects many types of mutations</td>
<td>Targeted to specific genes only. Time consuming and expensive</td>
<td>Commonly used to screen for specific genes causing disorders of hypothalamic-pituitary-GH axis</td>
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Table 2
Genetic technology used in the study of short stature
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<th>Gene panel testing</th>
<th>Next-generation sequencing for screening of multiple genes that may be causing the phenotype</th>
<th>Genetically heterogeneous disorders and overlapping phenotypes</th>
<th>Rapid and less expensive, can screen large numbers of genes efficiently</th>
<th>Needs Sanger confirmation</th>
<th>May identify variants of unknown significance. Cannot detect certain types of mutations</th>
<th>Genes can be selected based on phenotype to evaluate for isolated or syndromic short stature</th>
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<td>WES</td>
<td>Analysis of exons of the entire genome using high-throughput DNA sequencing technology</td>
<td>Genetic short stature, but no known single-gene defect is detected with other techniques, or not known</td>
<td>Identify cause of uncommon or rare conditions Efficient screening; targets only protein coding (exons) Efficient tool to identify pathologic variant in conditions with familial inheritance</td>
<td>Identifies variants of unknown significance. Cannot detect certain types of mutations</td>
<td>Variants in genes with unknown function may be detected. Incidental findings may be discovered in genes unrelated to phenotype Large data sets require ongoing standardization/ variant analyses remains a challenge</td>
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estimated, based on the rate of people having genetic disorders, that the molecular
diagnostic yield of WES is up to 25% compared with using a single-gene approach,
which yielded results in less than 15% of patients.55 Other investigators who have
used WES on adult patients have reported similar results, with a diagnostic rate of
17.5%.56 Reports have commented that the utility of this technology has helped to
produce unexpected findings of de novo mutations that would not have been identi-
fied with more traditional genetic screenings.

In endocrinology, there has been some application of WES in the study of disorders
such as type 2 diabetes mellitus, hypogonadism, and endocrine neoplasia.57 Mutations
within individual genes may play a major role in determining an individual’s height
and, with the assumption that unidentified highly penetrant genetic variants contribute
to stature, WES will continue to be an effective tool in advancing the field. In one recent
report, WES was performed on patients identified with severe short stature more than
3 SD less than the mean for age, and the investigators reported that, in 36% of the
families analyzed, a causal gene was identified.58 As with other forms of genetic anal-
ysis, the strategy of screening cohorts of families to help filter out normal variants may
help to better target more pathogenic variants.

One example of how WES has provided helpful insight into short stature is a recent
report that identified a gene that encodes aggrecan, a proteoglycan in the extracellular
matrix of growth plate and other cartilaginous tissues, known as ACAN. A heterozy-
gous gene mutation was identified in individuals with short stature along with an
advanced bone age through WES and predicted to adversely affect protein function.59
The described heterozygous mutations in ACAN seem to lead to mild skeletal
dysplasia.

As with other methods of genomic analysis, the limitations of WES include the
inability to recognize several potential genetic pathogenic causes of disease, including
important intronic regions or splice-site sequences, structural variants of DNA such as
translocations and inversions, genomic imprinting, epigenetic changes, and CNVs that
do not change DNA sequence. Furthermore, another limitation may be in the failure to
efficiently identify susceptibility genes in common disease traits, such as short stature,
that are complex and multifactorial. Susceptibility traits with generally low penetrance
and additive effects rarely are a primary cause of disorder. However, the continued
use of WES in clinical practice will inevitably provide better insights into rare,
higher-penetrance monogenic causes of short stature.

**SUMMARY**

It has been reported in a recent study that the standard medical evaluation for short
stature only had a 1.3% diagnostic yield for patients and thus shows the need to
expand diagnostic modalities for clinicians.9 The advances in understanding genetic
determinants of height will increase as researchers focus on specific phenotypes,
which could include the presentations of severe familial short stature, short stature
associated with specific syndromes, or children at risk for poor growth such as
SGA. Genetic tools and analyses are quickly revolutionizing the approach to under-
standing polygenic traits such as stature and will continue to assist in the identifica-
tion of monogenic disorders leading to abnormal stature.60 This research will inevitably
help to expand on the current limited therapies to treat short stature.61 Table 2 sum-
marizes several genetic techniques reviewed in this article, summarizing indications
and advantages for using each technique in the evaluation of stature. Such indications
and limitations apply to other areas of medicine as well.62,63 The endocrine evaluation
for stature will notably continue to evolve as technologies for genetic screening
improve. Each of the described techniques in this article has limitations in its ability to specifically target the causes of short stature, but each has helped to expand the appreciation of the complexities of height determination. The accessibility of patients to participate in research studies will increase and science will delineate the mechanisms of how genes determine stature. Once the mechanisms of disorders are better outlined, science will then be able to provide better diagnostic tools, along with novel therapeutic options, to address abnormal stature. Genetic screening options with better-defined algorithms will ultimately become part of a new standard approach for clinicians treating patients with short stature.

REFERENCES


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Q8  Please verify the structure of the headings and subheadings throughout.
Q9  Please verify that the sentence beginning “This technique has been used…” reads as intended.
Q10 Please verify that italics are used only in accordance with scientific convention, and that such use is consistent throughout.
Q11 Please verify that "growth hormone" is the intended expansion for the abbreviation "GH", as used later.
Q12 Please verify that all abbreviations that can be expanded have been expanded at first use in the text.
Q13 Please provide the expansion for the abbreviation "GHRH".
Q14 Please verify that “no case of GHRH gene mutation is recorded” reads as intended.
Q15 Please verify that the language editing preserves the intended meaning throughout.
Q16 Please verify the expansion for the abbreviation "OMIM".
Q17 Please provide the expansion for the abbreviation "ISS".
Q18 Please verify that the sentence beginning “Further support for the importance of…” reads as intended.
Q19 Please provide the expansion for the abbreviation "CGH".
Q20 Please note that "IGF1" has been changed to "IGF-1" throughout.
Q21 Please verify that “−3 SD less than the mean” reads as intended.
Q22 Please provide the volume number and page range for Ref. 56.
Q23 Please verify the abbreviation legend for Table 1 and provide the expansions for the abbreviations "Prl" and "rGH".

Please check this box or indicate your approval if you have no corrections to make to the PDF file

Thank you for your assistance.