International guideline on genetic testing of children with short stature

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Abstract

Short stature may be caused by a multitude of conditions including genetic and non-genetic causes. Over the last decade, advances in genetic sequencing technologies have revolutionized our understanding of the underlying physiology of growth and greatly increased our ability to identify genetic etiologies of short stature. The current guideline provides a general overview of the approach to the evaluation of a child with short stature followed by recommendations identifying factors in the medical and family history, physical examination, radiographic, and laboratory work up which increase the likelihood of identifying a genetic etiology. An algorithm is proposed for the genetic work up of individuals with short stature based on their clinical presentation. The risks and benefits of genetic testing are discussed as well.

Introduction

Over the past two decades, the advancement and increased availability of genomic sequencing tools have provided numerous clinically significant insights into the etiology of short stature (SS), transforming the diagnostic approach to growth disorders and a wide range of congenital conditions. This guideline offers recommendations on the diagnostic approach to children with SS, focusing on indications for using currently available genetic tools. Recommendations are partly based on a systematic review and meta-analysis of the literature [Scalco_SystRev_pending]. Definitions and abbreviations used in this paper are shown in **Boxes 1 and 2**.

The traditional definition of SS is based on a statistical cut-off, i.e., a height of more than 2 standard deviations below the mean for sex and age based on appropriate population reference data, commonly expressed as a height standard deviation score (HSDS) of <-2.0 (2.3rd percentile). In this guideline, the term SS also includes two other manifestations of growth failure, i.e., a decreasing height SDS over time (growth faltering) and a height SDS below the expected range based on the sex-adjusted mid-parental height SDS (target height, TH[1] or conditional TH (cTH)[2,3]).

Human height is a polygenic and heterogeneous trait, with its heritability reported to be approximately 80% based on estimates from twin studies[4]. Both rare and common genetic variants concurrently affect human height. According to genome-wide association studies (GWAS), a combination of >12,000 independent single nucleotide variants (SNVs) (generally with a population allele frequency >1%), clustered within >7,000 non-overlapping genomic segments, covering about 21% of the genome, determine an individual's height potential[5]. While each of these common genomic variants has a very small effect on one’s height (each contributing less than 2 mm), in aggregate, they can explain about half of the heritability and nearly half of overall phenotypic variation in height (reviewed in[6]). Additionally, rare variants with a larger impact on height variability (ranging from approximately 1-4 cm) also contribute to height determination in the general population[6,7].

In individuals with SS, the condition may result from a single pathogenic variant following a clear monogenic inheritance pattern, which is both necessary and sufficient to explain the observed phenotype. In other cases, it may be attributed to a combination of two or more rare variants (digenic or oligogenic inheritance) or the interaction of common variants in a classical polygenic manner[8,9,10].

It is commonly assumed that if a child's HSDS is close to (c)TH SDS, a polygenic etiology may be most likely, and this is considered a benign condition leading to an adult height that is close to target height[11]. However, monogenic causes can also be found in such patients, especially autosomal dominant gene variants if one parent is short[12]. Another benign condition associated with SS in childhood and early adolescence is slow maturation of the epiphyseal growth plates, which, if combined with late onset of puberty, is termed 'constitutional delay of growth and puberty (CDGP). This condition typically results in a normal adult height, but on average 1 SD below TH[13].

Methods

International Growth Genetics Guideline Consortium

The work on this guideline was initiated by the Clinical Practice Committee of the European Society of Paediatric Endocrinology (ESPE). First, a small steering committee (A.D., A.A.L.J., M.D., J.M.W. and S.C.) was set up to design the format of the guideline and invite the methodologist (O.M.D.) and pediatric endocrinologists and medical geneticists with special expertise in genetic testing of short children to participate in the International Growth Genetics Guideline Consortium (IGGGC) (Mid 2024). The Presidents of the European Society of Human Genetics and American College of Medical Genetics were informed. The consortium (n=34) consisted of 21 pediatric endocrinologists, 11 clinical geneticists, 1 clinical laboratory geneticist, and 1 clinical

epidemiologist/endocrinologist. ESPE was the only sponsor and funded all costs related to the initiative.

A subcommittee of IGGGC (O.M.D., A.D., J.M.W, O.N. and J.H.D., chaired by A.A.L.J.), in collaboration with staff members of A.A.L.J. and A.D. performed a systematic review on the clinical question: “What is the diagnostic yield of genetic testing in children with short stature, and how do various clinical features influence this yield?” The full results are reported in a separate publication [Scalco_SystRev_pending] and the main findings are discussed in this guideline.

Another subcommittee of IGGGC (J.A., J.B., P.C., Y.H.J, O.N., chaired by J.H.D.) performed a literature search on the question “What are the clinical consequences of genetic findings in children with isolated short stature?”, using ten prevalent genetic causes of children presenting with isolated SS. The results are included in the Supplementary Information for this guideline.

Based on the planned format of the guideline, nine working groups were formed, chaired by O.M.D., O.N., J.A., A.A.L.J., J.M.W., I.N., M.D., A.D. and A.L., to formulate draft recommendations and rationales. The reports of the working groups were combined into several consecutive versions of the guideline, which were discussed and revised electronically. During the process, all participants completed conflict of interest forms, summarized in **Competing Interests**. A semi-final version served as a discussion document for an 8-hour hybrid meeting in May 2025 with all available members of the consortium, where all recommendations and rationales were discussed and revised. Consensus was reached upon discussion and in some cases by voting. Minority positions were considered in the rationale behind recommendations.

Target Groups and Aims

The guideline has been developed for pediatric endocrinologists, adult endocrinologists, medical geneticists and general pediatricians who care for children with growth disorders. The

overall purpose of this guideline is to provide clinicians with practical guidance on the diagnostic approach to children with SS. In clinical practice, both the recommendations and the clinical judgement of treating physicians should be considered. Recommendations are not meant to replace clinical acumen and may need adaptation to local circumstances. We acknowledge that in low-resource settings, financial and other restrictions may prevent clinicians from adhering to the recommendations.

Summary of methods used for guideline development

For this guideline we used 'Recommendations, Assessment, Development, and Evaluation' (GRADE) as a methodological basis to inform the recommendations[14]. Recommendations were not only informed by the quality of the evidence, but also by potential desirable and undesirable effects, values and preferences[14,15]. National contexts were also considered.

The recommendations are worded as 'recommend' (strong recommendation) and 'suggest' (weak recommendation). The quality of evidence behind the recommendations is classified as very low (⨁◯◯◯), low (⨁⨁◯◯), moderate (⨁⨁⨁◯), and strong (⨁⨁⨁⨁). A strong recommendation implies that virtually all well-informed stakeholders—including clinicians, patients, and policymakers—are expected to favor the proposed course of action. In contrast, a weak recommendation indicates that although the majority may follow the suggested management, a notable proportion may reasonably opt for an alternative approach[16]. Statements derived primarily from clinical expertise and consensus within the working group, rather than from systematic evidence appraisal, are categorized as 'good clinical practice' and are not assigned a formal grade. Recommendations that lack both a clear evidence base and consensus-derived clinical rationale are not graded. Formal evidence assessment and grading were applied only to recommendations directly addressing the predefined clinical questions. The recommendations are divided into seven sections, as summarized in **Figure 1.**

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235 **Review process**

236 In October 2025, a draft of the guideline was reviewed by four experts in the field (see
237 'Acknowledgments' section), the Clinical Practice Committee of ESPE, various other regional societies
238 of pediatric endocrinology and various regional societies of human/medical genetics [including the
239 European Society of Human Genetics (ESHG) and American College of Medical Genetics (ACMG)] for
240 final approval and endorsement. All comments and suggestions were then discussed and
241 implemented as thought appropriate by the guideline working group (see **Supplementary Table 6**).
242 After incorporation of comments from the reviewers and various societies, all authors approved the
243 submitted version of the manuscript.

Recommendations

A. Recommendation regarding the use of a diagnostic classification of SS (R1)

R1. We suggest using a descriptive classification after the initial assessment of the child with SS, followed by an etiological classification after complete evaluation.

Rationale

The clinician is expected to have a basic knowledge of the most prevalent and clinically relevant causes of SS and the diagnostic work-up. At the initial assessment, SS can be stratified by subtype based on clinical evaluation. Categorization is important for the diagnostic process and often points towards a likely set of diagnoses. We suggest that children with SS are first classified according to the following clinical parameters: 1) Prenatal vs postnatal onset; 2) Skeletal malformation/disproportion vs no skeletal malformation/disproportion; 3) Presence or absence of syndromic characteristics (non-skeletal abnormalities); 4) Isolated SS vs non-isolated SS; and 5) Familial vs non-familial SS (**Supplementary Information 1, part 1**). Criteria for syndromic SS include clinical features such as microcephaly, congenital anomalies, facial dysmorphism, and developmental disorders (developmental delay, intellectual disability, autism spectrum disorder). When the diagnostic work-up is completed, the patient can be classified according to an etiological classification (**Supplementary Information 1, part 2**).

B. Recommendations on genetic investigation in clinical practice (R2-R7)

In recent decades, numerous molecular techniques have been developed to analyze genetic and epigenetic variants. Many of these have been incorporated into the clinical evaluation of patients suspected of a genetic condition, including children with SS. Clinicians must be familiar with the primary indications for each technique, as well as their limitations. They must also remain informed about regionally available genetic testing. **Table 1** summarizes the types of genetic variants

detected by currently available genetic diagnostic tools, including their limitations and comparative cost and highlights their applications. The availability and cost of genetic tests vary significantly between countries.

R2. We recommend close collaboration between clinical laboratory geneticists, medical geneticists and pediatric endocrinologists in the indication for genetic tests and interpretation of their results; genetic counseling is recommended for every family undergoing genetic testing. (Good clinical practice)

Rationale

Ideally, there is close collaboration between pediatric endocrinologists, pediatric radiologists, clinical laboratory geneticists and medical geneticists for interpretation of genetic testing results. A multidisciplinary clinic would be the ideal setting for communicating and discussing the results and implications of a genetic test with patients and their parents. The level of evidence of the association of a gene with a given phenotype is discussed in **Supplementary Information 2**.

R3. We recommend that variant pathogenicity is classified by the laboratory according to published guidelines (ACMG/ACGS). (Good clinical practice)

Rationale

Guidelines for genetic variant interpretation incorporate multiple lines of evidence[17]. It is essential that the classification of any identified variant is explicitly contextualized in relation to the relevant phenotype and mode of inheritance. This information should be clearly presented in the report to allow for clinical interpretation and appropriate medical decision-making (see **Supplementary Information 2**). However, for many variants identified in children with SS, it is difficult to definitively assign pathogenicity. Segregation analysis may be helpful (see **R4**) but is

confounded by multiple factors including assortative mating (the fact that short individuals tend to partner with other short individuals), incomplete penetrance, variable expressivity and the existence of phenocopies. Thus, *in vitro* functional characterization is an important adjunct tool to provide additional evidence whether a variant is pathogenic or not. This is not an easy task in the diagnostic setting but is important when treatment is available or the child may be able to participate in a clinical trial depending on the interpretation of the variant.

Over the last few years, diagnostic laboratories have started to perform rapid functional assays where the results can influence variant interpretation in the clinical report[18], although so far, this is rarely performed in clinical practice. These tests may include testing the effect of variants on splicing or the determination of a reduction or increase of RNA expression using quantitative real time PCR assays. These assays may only be feasible when the gene is expressed in easily accessible tissues such as blood or urine or, if necessary, skin biopsies.

Additionally, for certain conditions, it is possible to identify a characteristic methylation profile (DNA methylation signatures, **Table 1**) that defines the disease which would provide supportive evidence for variant pathogenicity[19], or gene expression signatures that can characterize a condition and indicate impact on functional pathways[20]. These DNA methylation/gene expression signatures (which are not variant-specific) provide a lower level of support for pathogenicity of a variant than variant-specific functional assays.

R4. We recommend that segregation analysis should be performed in patients where it may alter classification of the variant's pathogenicity. (Good clinical practice)

Rationale

Segregation analysis in parents and/or relatives can be helpful as a criterion for changing the classification of a variant of uncertain significance (VUS) to likely pathogenic or benign. Therefore, in such patients testing of other family members should be considered.

R5. We recommend that testing of other family members should be considered when the identification of the same pathogenic variant in relatives could influence healthcare management and/or enable more precise genetic counseling. (Good medical practice)

Rationale

The decision to pursue familial analysis should consider the specific gene involved, the predicted inheritance pattern, and the associated phenotypes. Diagnostic variant screening in children should only be conducted if it provides a potential health benefit for the child[21]. This process should always be preceded by thorough genetic counseling, including a discussion of the potential benefits, limitations, and projected outcomes of testing.

R6. If the patient develops new clinical features, re-analysis of available genetic data should be performed. In children with persistently unexplained SS in whom genetic testing was previously performed, we recommend that reanalysis of genetic data be considered periodically, taking into consideration bioinformatic improvements and new genetic discoveries. (Good clinical practice)

Rationale

Reanalyzing exome or genome data is recommended periodically due to the progression of genetic knowledge and technology[22,23]. Since the annotation of variants has improved after establishing vigorous QC measures for ES around 2018[24], resequencing should be considered for DNA samples tested before that time from patients with a high likelihood of a genetic cause but a previous negative test result. Additional resources for such re-evaluation of results over time should

be provided by payers. This recommendation is based on the potential for new gene-disease associations, refinements in classification of variants, and advancements in bioinformatics that can enhance diagnostic yield[25,26].

R7. We recommend that the benefits and risks of the genetic investigation in a child with SS should be carefully discussed with the family on an individual basis in a pre-test appointment. (Good clinical practice)

Rationale

Prior to embarking on genetic testing, one should carefully consider the potential benefits and risks from pursuing genetic investigations, summarized in **Box 3**. For a more detailed discussion on this topic, see **Supplementary Information 3** and the results of the literature review on the clinical consequences of 10 prevalent genetic causes encountered in children with isolated SS (**Supplementary Information 4**).

C. Recommendations regarding assessment of relevant diagnostic clues for a genetic cause of SS from the medical and family history (R8-R12)

In this and the three following sections (**Figure 1**), we present recommendations regarding diagnostic clues from the medical and family history (**section C, R8-12**), detailed medical examination (**section D, R13-16**), radiographs (**section E, R17-19**) and laboratory investigations (**section F, R20-22**) that have been associated with an increased likelihood of a genetic cause and/or indicate a specific genetic cause of SS. These findings may guide the choice of test and the interpretation of results. We also summarize the evidence on whether the presence of these clinical features in fact increases the diagnostic yield of genetic testing.

A proper medical assessment of a child with SS includes a detailed medical and family history, clinical examination, radiological assessment and laboratory investigation. This should assist the clinician in preparing a differential diagnosis ranked according to the likelihood of a primary or secondary growth disorder (intrinsic or extrinsic to the growth plate, respectively, **Supplementary Information 1**). For general characteristics of these categories, see **Supplementary Information 5 and 6.**

While for most secondary growth disorders monogenic causes are rare, a monogenic cause can be relatively frequently found in primary growth disorders. Thus, after exclusion of a non-genetic secondary growth disorder, the clinician faces the challenge of estimating the likelihood of a genetic cause of the patient's SS. All genetic syndromes associated with SS (6037 entries in OMIM, May 2025) are associated with their own phenotypic profiles. These phenotypes have been expanding with the increasing use of next-generation sequencing (NGS) identifying more mildly affected individuals, leading to numerous syndromes with partially overlapping phenotypes.

R8. We recommend searching for diagnostic clues for a primary or secondary growth disorder from the medical history of the child and family including a three-generation pedigree. (Good clinical practice)

Rationale

A thorough medical history (including review of systems) and family history of the short child can offer important clues to the etiology. Secondary growth disorders (**Supplemental Information 6**) can usually be suspected based on the clinical assessment and laboratory screening and confirmed through targeted laboratory testing. Identifying clinical information that increases the likelihood of a primary growth disorder of genetic origin (**Supplementary Information 5**) can help guide genetic testing.

R9. In children born SGA with persistent isolated or non-isolated SS for whom no cause could be identified, we recommend thorough clinical evaluation for imprinting disorders followed by specific DNA methylation testing where suspected. (⊕⊕◯◯)

Rationale

The underlying mechanism leading to being born SGA can involve maternal, placental, and/or fetal factors[27]. Therefore, SGA refers to a heterogeneous group of children with different etiologies and clinical outcomes. Most SGA-born children experience catch-up growth and achieve a height within their TH range, whereas approximately 10% have persistent SS ("short SGA")[28].

Children with short SGA and clinical features suggestive of an imprinting disorder (such as Silver-Russell syndrome or Temple syndrome) should be investigated by DNA methylation testing. The decision to test should be guided by the NH-CSS (Netchine-Harbison clinical scoring system). Initial testing should include methylation analysis of imprinted loci on chromosomes 11p15, 7 and 14q32[29,30] [Wakeling;inpreparation]. If negative, testing for alternative diagnoses (including variants in *IGF2*, *CDKN1C*, *HMGA2*, *PLAG1* or upd(20)mat) is recommended [Wakeling;inpreparation].

R10. In children born SGA with persistent isolated or non-isolated SS for whom no cause could be identified and in whom rhGH treatment is considered, we recommend comprehensive genetic testing for diagnostic purposes (see algorithm) and to identify rare genetic conditions in which rhGH treatment is contraindicated. (⊕⊕⊕◯)

As some etiologies of short SGA may increase cancer risk due to defects in DNA damage repair or replication[31], it is important to clinically evaluate all children with unexplained short SGA and perform genetic testing prior to rhGH initiation, especially when associated with microcephaly, dysmorphic features, developmental delay and/or learning disability.

In a child with isolated SS, SGA status does not increase the likelihood of identifying a genetic etiology [ScalcoSystRevpending]. However, many children with syndromic growth disorders may also be born SGA leading to higher rates of genetic diagnoses in the larger short SGA population[32]. An estimate of the diagnostic yield of genetic testing in short SGA through a conventional literature review is shown in **Supplementary Information 7**. The current list of genetic causes associated with SS and increased cancer risk is presented in **Supplementary Information 8**. In such patients the risks and benefits should be carefully weighed and discussed thoroughly with the patient allowing for shared decision making as to whether to proceed with rhGH treatment.

R11. We recommend genetic testing in a short child with major malformations and/or neurodevelopmental disorders. (⊕⊕⊕◯)

Rationale

With a detailed medical history, symptoms of any organ or system dysfunction (e.g., brain, heart, lung, kidneys, ears, eyes, skeleton, immune system, hemostasis) can be identified, and information can be collected on the presence of a neurodevelopmental disorder [developmental delay (DD), intellectual disability (ID) or neurological/behavioral symptoms, e.g., autism spectrum disorder]. A neurodevelopmental disorder is an established indication for genetic testing irrespective of height[33]. A search of the OMIM database identified 1,967 entries with SS and neurodevelopmental delay in the clinical synopsis (May 2025).

Based on the systematic review [ScalcoSystRevpending], the diagnostic yield of genetic testing is 15.1% (95% CI 10.4-20.6%) in isolated SS, 50.8% (43.1-58.4%) in syndromic SS including those with neurodevelopmental disorders and 69.8% (61.1-77.9%) in skeletal dysplasias. For an estimate of the diagnostic yield of genetic testing in children presenting with various other potential diagnostic clues see **Supplementary Information 9**.

R12. We recommend genetic testing in a short child if the family history suggests autosomal dominant, autosomal recessive, X-linked or mitochondrial inheritance, or if the child's height SDS is much shorter than that of both parents. (⊕⊕⊕◯)

Rationale

Evaluation of the inheritance pattern can help distinguish monogenic from polygenic causes. A three-generation pedigree, with information about parental consanguinity and heights of siblings, parents, grandparents, aunts and uncles, may help identify patterns of inheritance such as recessive, dominant, X-linked, or mitochondrial, or may raise the possibility of an imprinting disorder (for details, see **Supplementary Information 5 and 10**). In a child with no family history of SS, genetic etiologies should still be considered. However, the cause of mild familial SS in most individuals is likely polygenic[34].

An autosomal dominant growth disorder is suspected if one parent has a similar HSDS as the short child. As noted above, due to assortative mating, autosomal dominant growth disorders may also be found if both parents are short. Recessive growth disorders are more commonly found in consanguineous families or in small and isolated communities but should also be suspected in non-consanguineous families when two or more affected siblings are born to unaffected parents. If maternal-side male relatives are short and the patient is a boy, X-linked inheritance of SS should be suspected. If no other family members are affected, an autosomal recessive, X-linked recessive, or *de novo*-dominant inheritance should be considered.

Five studies have shown that having a family history of SS represented by at least one parent with height SDS < -2 significantly increases the diagnostic yield [ScalcoSystRevpending].

D. Recommendations regarding assessment of relevant diagnostic clues for a genetic cause of SS from the physical examination (R13-16)

R13. We recommend performing a detailed clinical examination before referring for genetic testing. (Good clinical practice)

Rationale

A thorough physical examination (deep phenotyping) is essential in the clinical work-up of a child with SS. Diagnostic clues for primary and secondary disorders are summarized in **Supplementary Information 5 and 6**, respectively. The focus should be on the anthropometric assessment, which, in addition to height and weight measurements, should include head circumference, arm span, and sitting height. Pubertal stage should be evaluated. Assessing the presence of dysmorphic features, skin abnormalities, skeletal anomalies, and congenital malformations is also crucial for establishing clinical diagnoses, guiding genetic studies, and identifying potential candidate genes.

R14. We recommend assessing for Turner syndrome including its mosaic form by a validated genetic test in a girl with clinical features suggestive of Turner syndrome, as well as in any girl with unexplained SS. (⊕⊕⊕⊕)

Rationale

In textbooks and guidelines, it has been advised that karyotyping should be performed in all girls with unexplained SS. This is based on observations that SS can be the only presenting sign of Turner syndrome and on the important clinical consequences of establishing the diagnosis[35]. If karyotyping is used, a minimum of 30 metaphases should be analyzed. Other validated methods besides karyotype may be employed in certified laboratories.

The diagnostic yield of this approach in girls with characteristic clinical features is assumed to be high. In contrast, the diagnostic yield of karyotyping in otherwise asymptomatic short girls has been reported as 2.5% in two small studies[36,37]. In a population-based epidemiological study the age-

and sex-specific cumulative incidences from birth until 16 years of age was 52 per 100 000 at 16 years[38].

R15. We recommend genetic testing in a short child if the auxological assessment shows one of the following: severe SS (height <-3 SDS); microcephaly; macrocephaly (absolute or relative); or body disproportion (Sitting height/height or arm span/height outside +/- 2.5 SDS). (⊕⊕⊕◯)

Rationale

Measurement of various auxological parameters (height, head circumference and body proportions) is essential in the assessment of a short child. Although no studies have been reported in which the impact of severe SS, microcephaly and disproportionate SS have been investigated in isolation, circumstantial evidence from the literature suggests that the diagnostic yield of genetic testing of SS is increased with increasing severity of shortness and additional clinical features (**Supplementary Information 5**).

Severe SS (height < -3 SDS)

While the presence of dysmorphic features or skeletal changes are probably the most important predictors of a genetic condition, the literature suggests that adults and children with severe isolated SS have an increased likelihood of establishing a genetic cause [ScalcoSystRevpending]. However, the presence of other clinical features in the reported patient cohorts[39,40] does not allow for accurate quantification of the effect of the severity of SS.

Microcephaly and relative macrocephaly

The presence of microcephaly in a short child may increase the diagnostic yield of genetic testing[41] and also points to specific etiologies, such as a heterozygous pathogenic *IGF1R* variant or a DNA repair syndrome[42]. In two studies (heterogeneous in terms of patient characteristics), the

presence of microcephaly in children with syndromic SS increased the diagnostic yield from 44% to 56.3%[41] and 24.5% to 83.3%[43].

Relative macrocephaly at birth is commonly seen in infants with Silver-Russell syndrome, Temple syndrome, 3M syndrome, and hypochondroplasia, among other genetic diseases. In most children with achondroplasia, relative macrocephaly progresses to true macrocephaly before the age of 2 years. No information is available on the impact of this feature on the diagnostic yield of genetic testing [ScalcoSystRevpending].

Disproportion

Several studies have reported that the presence of body disproportion increases the diagnostic yield of genetic testing in short children, particularly in genes responsible for skeletal disorders. Unfortunately, most of these reports did not define or quantify body disproportion[44,45,46,47]. Body disproportion may become more evident as the child ages.

In short children with mild skeletal anomalies, significant differences were observed for sitting height/height (SH/H) SDS in patients with an identified pathogenic variant in bone dysplasia associated genes (i.e., *ACAN*, *IHH*) compared to those without[48]. In short children tested for *SHOX*, a SH/H ratio SDS >2[49,50,51] or a SH/H >1 SDS or arm span ≥3 cm below height[52] appear to be useful predictive factors. In three studies focused on single genes involved in growth plate cartilage regulation—*SHOX* (in two studies) and *NPR2* (in one)—an elevated SH/H SDS (> +2) was associated with a marked increase in diagnostic yield. Reported yields rose from 3.1% to 13.8%[50], 5.7% to 40%[53], and 17.6% to 28.3%[51], respectively [ScalcoSystRevpending].

R16. We recommend genetic testing in a short child with clinical features suggestive of an underlying syndromic condition. (⊕⊕⊕⊕)

Rationale

Several studies have reported an increased diagnostic yield in short children who show features suggestive of a broader syndromic (genetic) disorder, either identified while taking the medical history (e.g., neurodevelopmental disorders) or after physical examination (facial dysmorphism and/or one or more congenital malformations, e.g., congenital heart disease) [ScalcoSystRevpending]. For example, in a large cohort of 304 patients with SS who underwent ES, those with syndromic features (defined as a systemic abnormality) as compared to those with isolated SS had a higher yield of genetic diagnoses (34% vs 11%)[54]. In short SGA children, a prominent forehead and triangular face point to Silver-Russell syndrome[55].

E. Recommendations regarding assessment of relevant diagnostic clues for a genetic cause of SS from the radiographic assessment (R17-19)

R17. We recommend performing a radiograph of the hand and wrist for bone age (BA) assessment in any child presenting with SS. Hand and wrist radiographs allow for identification of anatomic variants which may guide genetic investigation. (Good clinical practice)

Rationale

A radiograph of the (left) hand and wrist provides information on the degree of BA delay or advancement. A delayed BA is typical for a secondary growth disorder (e.g., juvenile hypothyroidism or growth hormone deficiency (GHD)) or for a general maturational delay, which may later present with delayed pubertal onset (then termed CDGP), considered a subclass of idiopathic short stature[3]. BA has limited utility below the age of 3 years.

Most primary growth disorders present with a normal or delayed BA, but in prepubertal children with heterozygous pathogenic variants in *ACAN*[56] or *GNAS*[57] an advanced BA is frequently observed.

The same radiograph can also provide information about anatomical abnormalities associated with genetic disorders. This can guide genetic investigations, particularly in children with isolated SS, who may carry defects in genes associated with growth plate function[58]. For example, the

presence of a Madelung deformity is suggestive of *SHOX* haploinsufficiency. However, the radiological indications of skeletal dysplasia can be subtle, often making it difficult to recognize relatively mild forms of genetic skeletal disorders[59]. For examples, see **Supplementary Information 11**.

R18. We recommend performing a skeletal survey (a series of radiographs that examine representative parts of the skeleton) in short children suspected of having a skeletal disorder, especially in the presence of disproportionate SS, bone deformities or bone mineralization abnormalities.

Rationale

The evaluation of skeletal surveys in childhood in combination with other clinical findings (e.g., clinical photographs and growth charts), should ideally be performed by an experienced pediatric radiologist or clinician trained to recognize the characteristic radiographic patterns associated with a specific skeletal dysplasia or group of skeletal disorders[60,61,62]. Specific genetic skeletal disorders can often be suggested by particular radiographic findings, but the final diagnosis should be confirmed by genetic testing.

To date, more than 770 distinct genetic skeletal disorders have been described, which may result in various anomalies in the shape and size of specific bones in the skeleton[60]. Good clinical indicators for a skeletal dysplasia include disproportionate SS, brachydactyly, pathological fractures, cranial nerve palsies (in absence of a neuromuscular disorder), limb asymmetry, severe/progressive kyphoscoliosis, restricted or increased joint mobility and waddling gait.

The following X-rays are recommended for a comprehensive survey, but a more tailored approach may be warranted in certain situations: anterior-posterior (AP) and lateral view radiographs of the skull and spine, AP views of the pelvis and all four extremities (unilateral, if no asymmetry), and AP views of the hands and feet. The radiation dose of such skeletal survey is

583 relatively low[63]. To further minimize the effects of radiation in the newborn, a "babygram" (AP and
584 lateral views) is advised[64].
585
586 **R19.** We recommend genetic testing in a short child with clinical or radiographic skeletal
587 abnormalities. (⨁⨁⨁⨁)
588 *Rationale*
589 The presence of clinical or radiological skeletal abnormalities (**Supplemental Information 11**)
590 increases the diagnostic yield of genetic testing in short children[39,65,41,66,67][ScalcoSystRevpending].
591 For details of skeletal findings associated with specific skeletal dysplasias, see Spranger et al[68].
592
593 **F. Recommendations regarding assessment of relevant diagnostic clues for a genetic cause of SS**
594 **from laboratory investigations (R20-22)**
595
596 **R20.** We recommend that each child with SS should undergo a laboratory evaluation, either as a
597 screening procedure or guided by clinical features. (Good clinical practice)
598 *Rationale*
599 The purpose of laboratory evaluation in short children, either in the form of a standardized
600 screening or guided by clinical features, is to detect indications of a primary or secondary growth
601 disorder. Similarly to any other screening procedure, the benefit of diagnosing a treatable condition
602 at an early stage (effectiveness) has to be weighed against the costs. Pediatric textbooks and
603 guidelines have suggested that laboratory screening of short children should be performed by a
604 general pediatrician so that easily diagnosable and treatable conditions (e.g., hypothyroidism, celiac
605 disease) would be detected and treated as early as possible[3,69]. Others have suggested that
606 laboratory tests should be guided by clinical features rather than routinely applied to all patients

607 with SS[70]. A list of potentially useful laboratory screening tests is shown in **Supplemental Information**
608 **12**.

609

610 **R21.** We recommend genetic testing using next-generation sequencing (ES/GS or a targeted gene
611 panel in a short child with severe GHD and/or anatomical abnormalities of the
612 hypothalamus/pituitary area known to be associated with genetic causes. (Good clinical practice)

613 *Rationale*

614 GHD may be isolated (IGHD) or combined with other hormone deficiencies (combined
615 pituitary hormone deficiency, CPHD). Both belong to a spectrum of disorders under the umbrella of
616 congenital hypopituitarism (CH), a heterogeneous and complex disorder, associated with highly
617 variable clinical phenotypes ranging in severity. Over the last four decades, pathogenic variants have
618 been identified in numerous genes encoding hormones and their receptors, or developmental
619 proteins including transcription factors implicated in hypothalamo-pituitary (HP) development[71,72,73].

620 Affected patients manifest different CH phenotypes, CPHD and IGHD being the most
621 frequent. Less common phenotypes include septo-optic dysplasia (SOD) and holoprosencephaly
622 (HPE) (**Supplementary Information 13, Supplementary Table 3**). Whilst some of the variants show
623 classical autosomal recessive, autosomal dominant, and X-linked recessive inheritance, many of the
624 variants are monoallelic and associated with variable penetrance. Carriers of the variant, often in the
625 same family as the index patients, may manifest no or a milder clinical phenotype than the proband.
626 We therefore recommend caution in interpretation of genetic findings that are not recessively
627 inherited, particularly novel variants identified in genes with variable penetrance (see
628 **Supplementary Information 13**).

629 **Supplementary Table 4** summarizes the genes currently associated with CH and their mode
630 of inheritance. The clinical and neuroimaging phenotypes associated with CH are extremely

heterogeneous, with unpredictable endocrine deficiencies often evolving over time, particularly in patients with SOD or pituitary stalk interruption syndrome (PSIS)[74,75], making monitoring challenging and treatment complicated. Due to the increasing number of CH-related genes and the variability in phenotypes, next generation sequencing (ES/GS or a panel-based approach) is currently the most efficient approach in identifying causative pathogenic variants and investigating the possibility of oligogenicity[76,77].

Establishing the genetic cause of CH can have important clinical benefits. For example, the identification of Type 2 GHD due to an autosomal dominant pathogenic *GH1* variant should make the clinician aware of the potential appearance of other pituitary hormone deficiencies (ACTH, TSH and gonadotropins)[78]. Additionally, the identification of pathogenic variants in *PROP1* in patients with a pituitary mass can avert surgery as this mass is likely to involute at a later stage[79]**.** Further, a mild "partial isolated GHD" (MIM 615925), characterized by slow growth and low, borderline or even normal serum GH responses to a GH stimulation test, can be caused by a mono-allelic pathogenic *GHSR* variant. Such patients show adequate catch-up growth on rhGH treatment[80] .

R22. We recommend a targeted gene panel or first-line candidate gene approach in the short child with characteristic clinical and laboratory features of insensitivity to growth hormone or IGF-1. (⊕⊕◯◯)

Rationale

SS due to GH-IGF-1 axis defects is associated with varying degrees of GH insensitivity (GHI). Some of these (e.g., *IGF1R*) present with IGF-1 insensitivity. The clinical features range from extreme pre- and post-natal growth failure with other physical and laboratory abnormalities to milder clinical phenotypes (**Supplemental Information 14**). Many other genetic SS syndromes can also present with features of GHI, for example RASopathies[81,82]. Furthermore, a similar clinical presentation (mild

655 growth failure in combination with a borderline low serum IGF-1 and a normal serum GH response to
656 a GH stimulation test) can also be caused by conditions with normal GH sensitivity[80,83,84].

657 Developing a detailed pedigree is mandatory (**R8**), as genetic cases may be autosomal
658 recessive or dominantly inherited. When the physical examination, laboratory assessment and
659 radiological findings are consistent with a severe, 'classical' or typical, GHI presentation (decreased
660 serum IGF-1 and normal or high result of a GH stimulation test[85,86], a targeted gene panel approach
661 is recommended, including *GHR*, *STAT5B*, *STAT3*, *IGFALS*, *PAPPA2*, *IGF1*, *QSOX2*. Patients with
662 heterozygous defects of *IGF1R* or carrying a 15q26.3 deletion are usually born SGA and present with
663 (relatively) low head circumference and (relatively) high serum IGF-1, especially during GH
664 treatment[87].

665 A milder or atypical GHI phenotype makes clinical diagnosis more difficult. ES/GS allows
666 testing a broader range of genes, along with the potential for novel gene discovery. Less than half of
667 atypical GHI patients are genetically confirmed via targeted gene panel testing[88] indicating that a
668 broader short stature gene panel may be more cost-effective. Additional information is provided in
669 **Supplementary Information 14**, including **Supplementary Table 5**.

670

671 **G. General recommendations regarding positive and negative indications to perform genetic**
672 **testing in children with SS (R23-24)**

673 **R23.** We recommend that genetic testing for SS (beyond laboratory screening including testing for
674 Turner syndrome in girls) is **not** indicated in a child with isolated SS suspected of constitutional delay
675 of growth and puberty (CDGP) or with a strong suspicion of a polygenic origin. (Good clinical
676 practice).

677 *Rationale*

Children who present with mild to moderate SS (a height SDS -2 to -2.5 SDS), slow growth, delayed BA but a growth trajectory within the TH range when corrected for BA can be considered "slow maturers". There is often a family history of pubertal delay. These patients often show delayed pubertal onset in adolescence (females >13 years, males >14 years) and are subsequently labelled as CDGP. Since slow growth and delayed or absent puberty are also characteristic signs of Turner syndrome, this should be excluded before the diagnosis of CDGP in a girl can be accepted (see **R14**). By definition, CDGP is a diagnosis of exclusion. Once puberty has started, growth progresses normally and may also be prolonged. Several genes have shown to be associated with pubertal timing[89].

Currently, a polygenic origin of SS cannot yet be confirmed in the clinic, but we postulate that the likelihood of a monogenic cause in a child with mild and isolated SS with borderline short and non-syndromic parents, no indication of autosomal dominant inheritance, and a height SDS close to the target height SDS is low (<10%). We assume that a polygenic origin is more likely in such patients.

R24. We recommend considering genetic testing in any child with SS in whom information from personal and family medical history, physical examination, radiological or laboratory findings suggests an increased likelihood of a genetic cause (defined as a monogenic condition, chromosomal aberration, CNV or methylation disorder, not a polygenic origin). (⊕⊕⊕⊕)

Rationale

Each child presenting with SS deserves a full medical assessment, with special attention to all known diagnostic clues for a primary or secondary growth disorder. Current literature suggests that in children in whom a non-genetic growth disorder has been excluded and who present with one or more clinical or laboratory features known to increase the likelihood of a genetic cause, the diagnostic yield of genetic testing is sufficient to warrant genetic testing [ScalcoSystRevpending].

Genes with the strongest evidence of association with isolated SS in the absence of other specific clinical findings are *ACAN*, *COL2A1*, *FBN1*, *FGFR3*, *GH1*, *GHR*, *GHSR*, *IGF1R*, *IHH*, *NF1*, *NPR2*, *PTPN11*, and *SHOX* [ScalcoSystRevpending]. This can thus be considered a minimum list of genes recommended for evaluation in children with isolated SS. Depending on the expertise of each center and advances in the field, additional genes may be considered. Variants in genes typically associated with syndromic SS or skeletal dysplasia should be interpreted with caution in patients lacking characteristic features.

Figure 2 shows the algorithm summarizing this recommendation. Genome sequencing (short read or long read) is the standard approach in a number of countries, and we expect that to increase in the future, thus making the use of targeted gene panel testing obsolete. We recognize that in resource limited countries genetic investigations may not be available nor reimbursed.

Future perspectives

NGS, with the use of large gene panels, ES and GS, has revolutionized the diagnostic approach to the short child with SS as it has in many other areas of medicine. However, ES provides information only on protein-coding genes which correspond to approximately 2% of the genome. Genetic testing can currently identify a monogenic cause in fewer than 15% of children with isolated SS, whereas up to 80% of children with syndromic SS or suspected skeletal dysplasia may receive a genetic diagnosis [ScalcoSystrevpending]. Therefore, there are still further genes or novel genetic variants causing SS to be identified.

The limitations of current genetic testing will inevitably lead to applying GS in the near future to increase detection sensitivity for causative genetic variants. A recent study on genomes of a large cohort of families with suspected rare monogenic diseases has shown an incremental diagnostic yield of GS of approximately 8% for those who had previously undergone ES[90]. The main limitations to the large-scale use of GS are the higher analytical burden due to the millions of noncoding and structural

variants that can be identified. Previously, high cost was also a limitation, but currently the cost of the combination of ES and CMA is similar to the cost of GS, so that several laboratories are currently using GS as a first-line test[91]. We expect that the progressive use of artificial intelligence and reduction of costs will lead to more widespread use of GS as a first-line single test. In the future, we anticipate that the integration of multi-omic approaches facilitated by long-read sequencing will allow for the identification of additional genetic etiologies of growth disorders. As these approaches are integrated into clinical practice, diagnostic rates will improve[92].

With the growing discovery of regulatory and non-coding variants, understanding their transcriptomic impact will become increasingly important. We anticipate that RNA-seq will be incorporated into clinical practice to understand the potential impact of genetic variants on gene (mRNA) expression and that methylation signatures may play an increasing role in identifying genetic syndromes.

Digenic or oligogenic inheritance, where interaction of two or more genes located at different loci are observed, may account for a non-conventional pattern of inheritance underlying some forms of SS[93], as shown in a subset of patients with Noonan syndrome[94]. A systematic search for the phenotype resulting from the interplay between two or more genetic variants (epistasis) has become feasible only with modern machine learning methods[95]. The importance of the multiple gene effect on the growth process has been further emphasized by the development of polygenic risk scores for predicting familial SS[96,97] and adult height[98], showing an accuracy of 0.84-0.94. A polygenic risk score may help distinguish children with a benign, polygenic predisposition to short stature[97] and also identify those who may have an underlying monogenic cause[98].

In addition to sequence variants causing growth disorders, there is mounting evidence that epigenetic changes play a major role in the growth process. Epigenetic changes may directly affect transcriptional machinery or cause alterations in chromatin structure, making chromatin less or not accessible to transcription factors. The epigenetic processes that stably alter gene expression

752 patterns (and/or transmit the alterations at cell division) include DNA (cytosine) methylation, post-
753 translational modification of histone proteins and remodeling of chromatin, and RNA-based
754 mechanisms. Each of these epigenetic changes may have an impact on growth and are discussed in
755 **Supplementary Information 15**. Undoubtedly, with ongoing advances in genetic investigative
756 technologies, the importance of genetic testing in the diagnostic workup of short stature will
757 continue to increase.

758

References

1. Tanner JM, Goldstein H, Whitehouse RH. Standards for Children's Height at Age 2 to 9 years allowing for height of Parents. *Arch Dis Child*. 1970;45(244):819-819. doi:10.1136/ADC.45.244.819-B

2. Hermanussen M, Cole J. The calculation of target height reconsidered. *Horm Res*. 2003;59(4):180-183. doi:10.1159/000069321

3. Cohen P, Rogol AD, Deal CL, et al. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab*. 2008;93(11):4210-4217. doi:10.1210/JC.2008-0509

4. Jelenkovic A, Sund R, Hur YM, et al. Genetic and environmental influences on height from infancy to early adulthood: An individual-based pooled analysis of 45 twin cohorts. *Sci Rep*. 2016;6. doi:10.1038/SREP28496

5. Yengo L, Vedantam S, Marouli E, et al. A saturated map of common genetic variants associated with human height. *Nature*. 2022;610(7933):704-712. doi:10.1038/S41586-022-05275-Y

6. Bicknell LS, Hirschhorn JN, Savarirayan R. The genetic basis of human height. *Nat Rev Genet*. 2025;26(9). doi:10.1038/S41576-025-00834-1

7. Grunauer M, Jorge AAL. Genetic short stature. *Growth Hormone and IGF Research*. 2018;38:29-33. doi:10.1016/j.ghir.2017.12.003

8. Gropman AL, Adams DR. Atypical Patterns of Inheritance. *Semin Pediatr Neurol*. 2007;14(1):34-45. doi:10.1016/j.spen.2006.11.007

9. Deltas C. Digenic inheritance and genetic modifiers. *Clin Genet*. 2018;93(3):429-438. doi:10.1111/CGE.13150

10. Kingdom R, Wright CF. Incomplete Penetrance and Variable Expressivity: From Clinical Studies to Population Cohorts. *Front Genet*. 2022;13. doi:10.3389/FGENE.2022.920390

11. Rekers-Mombarg LTM, Wit JM, Massa GG, et al. Spontaneous growth in idiopathic short stature. European Study Group. *Arch Dis Child*. 1996;75(3):175-180. doi:10.1136/ADC.75.3.175

12. Plachy L, Strakova V, Elblova L, et al. High Prevalence of Growth Plate Gene Variants in Children With Familial Short Stature Treated With GH. *J Clin Endocrinol Metab*. 2019;104(10):4273-4281. doi:10.1210/jc.2018-02288

13. LaFranchi S, Hanna C, Mandel S. Constitutional delay of growth: expected versus final adult height. *Pediatrics*. 1991;87(1):82-87.

14. Langer G, Meerpohl JJ, Perleth M, Gartlehner G, Kaminski-Hartenthaler A, Schünemann H. GRADE Guidelines: 1. Introduction - GRADE evidence profiles and summary of findings tables. *Z Evid Fortbild Qual Gesundhwes*. 2012;106(5):357-368. doi:10.1016/j.zefq.2012.05.017

15. Dekkers OM, Burman P. ESE guidelines, why and how. *Eur J Endocrinol*. 2015;173(2):E1-E2. doi:10.1530/EJE-15-0625

16. Andrews JC, Schünemann HJ, Oxman AD, et al. GRADE guidelines: 15. Going from evidence to recommendation - Determinants of a recommendation's direction and strength. *J Clin Epidemiol*. 2013;66(7):726-735. doi:10.1016/j.jclinepi.2013.02.003

17. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*. 2015;17(5):405-423. doi:10.1038/gim.2015.30

18. Brnich SE, Rivera-Muñoz EA, Berg JS. Quantifying the potential of functional evidence to reclassify variants of uncertain significance in the categorical and Bayesian interpretation frameworks. *Hum Mutat*. 2018;39(11):1531-1541. doi:10.1002/HUMU.23609

19. Sadikovic B, Aref-Eshghi E, Levy MA, Rodenhiser D. DNA methylation signatures in mendelian developmental disorders as a diagnostic bridge between genotype and phenotype. *Epigenomics*. 2019;11(5):563-575. doi:10.2217/EPI-2018-0192

20. Cuvertino S, Garner T, Martirosian E, et al. Higher order interaction analysis quantifies coordination in the epigenome revealing novel biological relationships in Kabuki syndrome. *Brief Bioinform*. 2024;26(1). doi:10.1093/BIB/BBAE667

21. Ross LF, Saal HM, Anderson RR, David KL. Ethical and policy issues in genetic testing and screening of children. *Pediatrics*. 2013;131(3):620-622. doi:10.1542/PEDS.2012-3680

22. Costain G, Jobling R, Walker S, et al. Periodic reanalysis of whole-genome sequencing data enhances the diagnostic advantage over standard clinical genetic testing. *Eur J Hum Genet*. 2018;26(5):740-744. doi:10.1038/S41431-018-0114-6

23. Manickam K, McClain MR, Demmer LA, et al. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*. 2021;23(11):2029-2037. doi:10.1038/s41436-021-01242-6

24. Corominas J, Smeekens SP, Nelen MR, et al. Clinical exome sequencing-Mistakes and caveats. *Hum Mutat*. 2022;43(8):1041-1055. doi:10.1002/HUMU.24360

25. Wenger AM, Guturu H, Bernstein JA, Bejerano G. Systematic reanalysis of clinical exome data yields additional diagnoses: Implications for providers. *Genetics in Medicine*. 2017;19(2):209-214. doi:10.1038/gim.2016.88

26. Schobers G, Schieving JH, Yntema HG, et al. Reanalysis of exome negative patients with rare disease: a pragmatic workflow for diagnostic applications. *Genome Med*. 2022;14(1). doi:10.1186/S13073-022-01069-Z

27. Lee PA, Chernausek SD, Hokken-Koelega ACS, Czernichow P. International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. *Pediatrics*. 2003;111(6 Pt 1):1253-1261. doi:10.1542/PEDS.111.6.1253

28. Hokken-Koelega ACS, van der Steen M, Boguszewski MCS, et al. International Consensus Guideline on Small for Gestational Age: Etiology and Management From Infancy to Early Adulthood. *Endocr Rev*. 2023;44(3):539-565. doi:10.1210/ENDREV/BNAD002

29. Wakeling EL, Brioude F, Lokulo-Sodipe O, et al. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. *Nat Rev Endocrinol*. 2017;13(2):105-124. doi:10.1038/NRENDO.2016.138

30. Ogawa T, Narusawa H, Nagasaki K, et al. Temple Syndrome: Comprehensive Clinical Study in Genetically Confirmed 60 Japanese Patients. *J Clin Endocrinol Metab*. 2025;110(9). doi:10.1210/CLINEM/DGAE883

31. Giacomozzi C. Genetic Screening for Growth Hormone Therapy in Children Small for Gestational Age: So Much to Consider, Still Much to Discover. *Front Endocrinol (Lausanne)*. 2021;12. doi:10.3389/FENDO.2021.671361

32. Homma TK, Freire BL, Honjo Kawahira RS, et al. Genetic Disorders in Prenatal Onset Syndromic Short Stature Identified by Exome Sequencing. *J Pediatr*. 2019;215:192-198. doi:10.1016/J.JPEDS.2019.08.024

33. Srivastava S, Cole JJ, Cohen JS, et al. Survey of the Landscape of Society Practice Guidelines for Genetic Testing of Neurodevelopmental Disorders. *Ann Neurol*. 2024;96(5):900-913. doi:10.1002/ANA.27045

34. Silventoinen K, Sammalisto S, Perola M, et al. Heritability of adult body height: a comparative study of twin cohorts in eight countries. *Twin Res*. 2003;6(5):399-408. doi:10.1375/136905203770326402

35. Gravholt CH, Andersen NH, Christin-Maitre S, et al. Clinical practice guidelines for the care of girls and women with Turner syndrome. *Eur J Endocrinol*. 2024;190(6):G53-G151. doi:10.1093/EJENDO/LVAE050

36. Gicquel C, Gaston V, Cabrol S, Le Bouc Y. Assessment of Turner's syndrome by molecular analysis of the X chromosome in growth-retarded girls. *J Clin Endocrinol Metab*. 1998;83(5):1472-1476. doi:10.1210/JCEM.83.5.4805

37. Grandone A, Del Vecchio Blanco F, Torella A, et al. Multiplex Ligation-Dependent Probe Amplification Accurately Detects Turner Syndrome in Girls with Short Stature. *Horm Res Paediatr*. 2016;86(5):330-336. doi:10.1159/000452219

38. Harju S, Saari A, Sund R, Sankilampi U. Epidemiology of Disorders Associated with Short Stature in Childhood: A 20-Year Birth Cohort Study in Finland. *Clin Epidemiol*. 2022;14:1205-1214. doi:10.2147/CLEP.S372870

39. Huang Z, Sun Y, Fan Y, et al. Genetic Evaluation of 114 Chinese Short Stature Children in the Next Generation Era: a Single Center Study. *Cell Physiol Biochem*. 2018;49(1):295-305. doi:10.1159/000492879

40. Andrade NLM, Funari MF de A, Malaquias AC, et al. Diagnostic yield of a multigene sequencing approach in children classified as idiopathic short stature. *Endocr Connect*. 2022;11(12). doi:10.1530/EC-22-0214

41. Li X, Yao R, Chang G, et al. Clinical Profiles and Genetic Spectra of 814 Chinese Children With Short Stature. *J Clin Endocrinol Metab*. 2022;107(4):972-985. doi:10.1210/CLINEM/DGAB863

42. Juanes M, Guercio G, Marino R, et al. Three novel IGF1R mutations in microcephalic patients with prenatal and postnatal growth impairment. *Clin Endocrinol (Oxf)*. 2015;82(5):704-711. doi:10.1111/CEN.12555

43. Fang D, Li X, Zhang Z, et al. Clinical profiles and molecular genetic analyses of 98 Chinese children with short statures. *Front Genet*. 2024;15. doi:10.3389/FGENE.2024.1364441

44. Ezquieta B, Cueva E, Oliver A, Gracia R. SHOX intragenic microsatellite analysis in patients with short stature. *J Pediatr Endocrinol Metab*. 2002;15(2):139-148. doi:10.1515/JPEM.2002.15.2.139

45. Hirschfeldova K, Solc R, Baxova A, et al. SHOX gene defects and selected dysmorphic signs in patients of idiopathic short stature and Léri-Weill dyschondrosteosis. *Gene*. 2012;491(2):123-127. doi:10.1016/j.gene.2011.10.011

46. Plachy L, Dusatkova P, Maratova K, et al. Familial Short Stature-A Novel Phenotype of Growth Plate Collagenopathies. *J Clin Endocrinol Metab*. 2021;106(6):1742-1749. doi:10.1210/CLINEM/DGAB084

47. Gippert S, Wagner M, Brunet T, et al. Exome sequencing (ES) of a pediatric cohort with chronic endocrine diseases: a single-center study (within the framework of the TRANSLATE-NAMSE project). *Endocrine*. 2024;85(1):444-453. doi:10.1007/S12020-023-03581-7

48. Sentchordi-Montané L, Benito-Sanz S, Aza-Carmona M, et al. High prevalence of variants in skeletal dysplasia associated genes in individuals with short stature and minor skeletal anomalies. *Eur J Endocrinol*. 2021;185(5):691-705. doi:10.1530/EJE-21-0557

49. Jorge AAL, Souza SC, Nishi MY, et al. SHOX mutations in idiopathic short stature and Leri-Weill dyschondrosteosis: frequency and phenotypic variability. *Clin Endocrinol (Oxf)*. 2007;66(1):130-135. doi:10.1111/J.1365-2265.2006.02698.X

50. Malaquias AC, Scalco RC, Fontenele EGP, et al. The sitting height/height ratio for age in healthy and short individuals and its potential role in selecting short children for SHOX analysis. *Horm Res Paediatr*. 2013;80(6):449-456. doi:10.1159/000355411

51. Vannelli S, Baffico M, Buganza R, et al. SHOX deficiency in children with growth impairment: evaluation of known and new auxological and radiological indicators. *Ital J Pediatr*. 2020;46(1). doi:10.1186/S13052-020-00927-Z

52. Joustra SD, Kamp GA, Stalman SE, et al. Novel Clinical Criteria Allow Detection of Short Stature Homeobox-Containing Gene Haploinsufficiency Caused by Either Gene or Enhancer Region Defects. *Horm Res Paediatr*. 2019;92(6):372-381. doi:10.1159/000507215

53. Plachy L, Dusatkova P, Maratova K, et al. NPR2 Variants Are Frequent among Children with Familiar Short Stature and Respond Well to Growth Hormone Therapy. *J Clin Endocrinol Metab*. 2020;105(3). doi:10.1210/CLINEM/DGAA037

54. Fan X, Zhao S, Yu C, et al. Exome sequencing reveals genetic architecture in patients with isolated or syndromic short stature. *J Genet Genomics*. 2021;48(5):396-402. doi:10.1016/J.JGG.2021.02.008

55. Eggermann T, Gonzalez D, Spengler S, Arslan-Kirchner M, Binder G, Schönherr N. Broad clinical spectrum in Silver-Russell syndrome and consequences for genetic testing in growth retardation. *Pediatrics*. 2009;123(5). doi:10.1542/PEDS.2008-3228

56. Gkourogianni A, Andrew M, Tyzinski L, et al. Clinical Characterization of Patients With Autosomal Dominant Short Stature due to Aggrecan Mutations. *J Clin Endocrinol Metab*. 2017;102(2):460-469. doi:10.1210/jc.2016-3313

57. Mantovani G, Bastepe M, Monk D, et al. Diagnosis and management of pseudohypoparathyroidism and related disorders: first international Consensus Statement. *Nat Rev Endocrinol*. 2018;14(8):476-500. doi:10.1038/S41574-018-0042-0

58. Vasques GA, Andrade NLM, Jorge AAL. Genetic causes of isolated short stature. *Arch Endocrinol Metab*. 2019;63(1):70-78. doi:10.20945/2359-3997000000105

59. Chavhan GB, Miller E, Mann EH, Miller SF. Twenty classic hand radiographs that lead to diagnosis. *Pediatr Radiol*. 2010;40(5):747-761. doi:10.1007/S00247-009-1520-2

60. Unger S, Ferreira CR, Mortier GR, et al. Nosology of genetic skeletal disorders: 2023 revision. *Am J Med Genet A*. 2023;191(5):1164-1209. doi:10.1002/AJMG.A.63132

61. Handa A, Grigelioniene G, Nishimura G. Skeletal Dysplasia Families: A Stepwise Approach to Diagnosis. *Radiographics*. 2023;43(5):1-20. doi:10.1148/RG.220067

62. Alanay Y, Lachman RS. A review of the principles of radiological assessment of skeletal dysplasias. *J Clin Res Pediatr Endocrinol*. 2011;3(4):163-178. doi:10.4274/JCRPE.463

63. Sait S, Havariyoun G, Newman H, Das S, Haque S. Effective radiation dose of skeletal surveys performed for suspected physical abuse. *Pediatr Radiol*. 2023;53(1):69-77. doi:10.1007/S00247-022-05477-6

64. Yu CC. Radiation safety in the neonatal intensive care unit: Too little or too much concern? *Pediatr Neonatol*. 2010;51(6):311-319. doi:10.1016/S1875-9572(10)60061-7

65. Xiao H, Chen H, Chen X, et al. Comprehensive assessment of the genetic characteristics of small for gestational age newborns in NICU: from diagnosis of genetic disorders to prediction of prognosis. *Genome Med*. 2023;15(1). doi:10.1186/S13073-023-01268-2

66. Lin L, Li M, Luo J, et al. A High Proportion of Novel ACAN Mutations and Their Prevalence in a Large Cohort of Chinese Short Stature Children. *J Clin Endocrinol Metab*. 2021;106(7):E2711-E2719. doi:10.1210/CLINEM/DGAB088

67. Kim SJ, Joo E, Park J, Seol CA, Lee JE. Genetic evaluation using next-generation sequencing of children with short stature: a single tertiary-center experience. *Ann Pediatr Endocrinol Metab*. 2024;29(1):38-45. doi:10.6065/APEM.2346036.018

68. Spranger JW, Brill PW, Hall C, Nishimura G, Superti-Furga A, Unger S. *Bone Dysplasias*. Oxford University Press; 2018. doi:10.1093/med/9780190626655.001.0001

69. Wit JM, Kamp GA, Oostdijk W. Towards a Rational and Efficient Diagnostic Approach in Children Referred for Growth Failure to the General Paediatrician. *Horm Res Paediatr*. 2019;91(4):223-240. doi:10.1159/000499915

70. Collett-Solberg PF, Ambler G, Backeljauw PF, et al. Diagnosis, Genetics, and Therapy of Short Stature in Children: A Growth Hormone Research Society International Perspective. *Horm Res Paediatr*. Published online September 12, 2019:1-14. doi:10.1159/000502231

71. Kelberman D, Rizzoti K, Lovell-Badge R, Robinson ICAF, Dattani MT. Genetic regulation of pituitary gland development in human and mouse. *Endocr Rev*. 2009;30(7):790-829. doi:10.1210/ER.2009-0008

72. Fang Q, George AS, Brinkmeier ML, et al. Genetics of Combined Pituitary Hormone Deficiency: Roadmap into the Genome Era. *Endocr Rev*. 2016;37(6):636-675. doi:10.1210/ER.2016-1101

73. Gregory LC, Dattani MT. The Molecular Basis of Congenital Hypopituitarism and Related Disorders. *J Clin Endocrinol Metab*. 2020;105(6). doi:10.1210/CLINEM/DGZ184

74. Cerbone M, Dattani MT. Progression from isolated growth hormone deficiency to combined pituitary hormone deficiency. *Growth Hormone and IGF Research*. 2017;37:19-25. doi:10.1016/j.ghir.2017.10.005

75. Cerbone M, Güemes M, Wade A, Improda N, Dattani M. Endocrine morbidity in midline brain defects: Differences between septo-optic dysplasia and related disorders. *EClinicalMedicine*. 2020;19. doi:10.1016/j.eclinm.2019.11.017

76. Simm F, Griesbeck A, Choukair D, et al. Identification of SLC20A1 and SLC15A4 among other genes as potential risk factors for combined pituitary hormone deficiency. *Genetics in Medicine*. 2018;20(7):728-736. doi:10.1038/gim.2017.165

77. Martinez-Mayer J, Vishnopolska S, Perticarari C, et al. Exome Sequencing Has a High Diagnostic Rate in Sporadic Congenital Hypopituitarism and Reveals Novel Candidate Genes. *J Clin Endocrinol Metab*. 2024;109(12):3196-3210. doi:10.1210/CLINEM/DGAE320

78. Mullis PE, Robinson ICAF, Salemi S, et al. Isolated autosomal dominant growth hormone deficiency: an evolving pituitary deficit? A multicenter follow-up study. *J Clin Endocrinol Metab*. 2005;90(4):2089-2096. doi:10.1210/JC.2004-1280

79. Turton JPG, Mehta A, Raza J, et al. Mutations within the transcription factor PROP1 are rare in a cohort of patients with sporadic combined pituitary hormone deficiency (CPHD). *Clin Endocrinol (Oxf)*. 2005;63(1):10-18. doi:10.1111/J.1365-2265.2005.02291.X

80. Punt LD, Kooijman S, Mutsters NJM, et al. Loss-of-Function GHSR Variants Are Associated With Short Stature and Low IGF-I. *J Clin Endocrinol Metab*. 2025;110(5):e1303-e1314. doi:10.1210/CLINEM/DGAF010

81. Malaquias AC, Jorge AAL. Activation of the MAPK pathway (RASopathies) and partial growth hormone insensitivity. *Mol Cell Endocrinol*. 2021;519. doi:10.1016/J.MCE.2020.111040

82. Andrews A, Maharaj A, Cottrell E, et al. Genetic Characterization of Short Stature Patients With Overlapping Features of Growth Hormone Insensitivity Syndromes. *J Clin Endocrinol Metab*. 2021;106(11):E4716-E4733. doi:10.1210/CLINEM/DGAB437

83. Wit JM, Joustra SD, Losekoot M, Van Duyvenvoorde HA, De Bruin C. Differential Diagnosis of the Short IGF-I-Deficient Child with Apparently Normal Growth Hormone Secretion. *Horm Res Paediatr*. 2021;94(3-4):81-104. doi:10.1159/000516407

84. Spiliotis BE, August GP, Hung W, Sonis W, Mendelson W, Bercu BB. Growth hormone neurosecretory dysfunction. A treatable cause of short stature. *JAMA*. 1984;251(17):2223-2230.

85. Storr HL, Chatterjee S, Metherell LA, et al. Nonclassical GH Insensitivity: Characterization of Mild Abnormalities of GH Action. *Endocr Rev*. 2019;40(2):476-505. doi:10.1210/ER.2018-00146

86. Savage MO, Storr HL, Backeljauw PF. The continuum between GH deficiency and GH insensitivity in children. *Rev Endocr Metab Disord*. 2021;22(1):91-99. doi:10.1007/S11154-020-09590-5

87. Walenkamp MJE, Robers JML, Wit JM, et al. Phenotypic Features and Response to GH Treatment of Patients With a Molecular Defect of the IGF-1 Receptor. *J Clin Endocrinol Metab*. 2019;104(8):3157-3171. doi:10.1210/JC.2018-02065

88. Cottrell E, Cabrera CP, Ishida M, et al. Rare CNVs provide novel insights into the molecular basis of GH and IGF-1 insensitivity. *Eur J Endocrinol*. 2020;183(6):581-595. doi:10.1530/EJE-20-0474

89. Howard SR. The Genetic Basis of Delayed Puberty. *Front Endocrinol (Lausanne)*. 2019;10(JUN). doi:10.3389/FENDO.2019.00423

90. Wojcik MH, Lemire G, Berger E, et al. Genome Sequencing for Diagnosing Rare Diseases. *N Engl J Med*. 2024;390(21):1985-1997. doi:10.1056/NEJMOA2314761

91. Runheim H, Pettersson M, Hammarsjö A, et al. The cost-effectiveness of whole genome sequencing in neurodevelopmental disorders. *Sci Rep*. 2023;13(1). doi:10.1038/S41598-023-33787-8

92. Vollger MR, Korlach J, Eldred KC, et al. Synchronized long-read genome, methylome, epigenome and transcriptome profiling resolve a Mendelian condition. *Nat Genet*. 2025;57(2):469-479. doi:10.1038/S41588-024-02067-0

93. Okazaki A, Ott J. Machine learning approaches to explore digenic inheritance. *Trends in Genetics*. 2022;38(10):1013-1018. doi:10.1016/j.tig.2022.04.009

94. Ferrari L, Mangano E, Bonati MT, et al. Digenic inheritance of subclinical variants in Noonan Syndrome patients: an alternative pathogenic model? *Eur J Hum Genet*. 2020;28(10):1432-1445. doi:10.1038/S41431-020-0658-0

95. Elgart M, Lyons G, Romero-Brufau S, et al. Non-linear machine learning models incorporating SNPs and PRS improve polygenic prediction in diverse human populations. *Commun Biol*. 2022;5(1). doi:10.1038/S42003-022-03812-Z

96. Lin YJ, Cheng CF, Wang CH, et al. Genetic Architecture Associated With Familial Short Stature. *J Clin Endocrinol Metab*. 2020;105(6):1801-1813. doi:10.1210/CLINEM/DGAA131

97. Shelley JP, Shi M, Peterson JF, Van Driest SL, Simmons JH, Mosley JD. A polygenic score for height identifies an unmeasured genetic predisposition among pediatric patients with idiopathic short stature. *Genome Med*. 2025;17(1). doi:10.1186/S13073-025-01455-3

98. Lu T, Forgetta V, Richards JB, Greenwood CMT. Polygenic risk score as a possible tool for identifying familial monogenic causes of complex diseases. *Genetics in Medicine*. 2022;24(7):1545-1555. doi:10.1016/j.gim.2022.03.022

99. Miller DT, Adam MP, Aradhya S, et al. Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies. *Am J Hum Genet*. 2010;86(5):749-764. doi:10.1016/j.ajhg.2010.04.006

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Author contributions

A.D, A.A.L.J., S.C. and J.M.W. contributed to the general design of the guideline, the systematic review, and chaired a working group and engaged in all aspects of the article. J.M.W. coordinated and edited the consecutive versions of the manuscript. O.N. contributed to the systematic review, chaired a working group and contributed to all aspects of the article. J.A. and I.N. chaired a working group and contributed to all aspects of the article. M.D. contributed to the general design and chaired a working group. O.M.D. served as methodology lead for the design of the guideline and systematic review. P.B., J.B., D.B., P.C., J.H.D., T.Ed., T.Eg, E.G., G.G., K.H., Y.H.J., P.L, G.M., S.P., H.S., E.W., C.R.F., contributed to the first versions of working group reports and contributed to all aspects of the article. T.H., A.H-K, A.L., X.L., X.W., V.H., L.G. and F.B. contributed to the first versions of the working group reports.

Competing interests

A.D. reported receiving consulting fees and grants from Biomarin, BridgeBio, Novo Nordisk and Pfizer. A.A.L.J. reported receiving consulting fees and grants from BioMarin, Novo Nordisk and BridgeBio. O.N. reported serving as principal investigator in sponsored trials of Ascendis and Alexion, receiving consulting fees from Kyowa Kirin and speaker's honoraria from MSD. I.N. reported receiving a research grant from Merck and Pfizer and travel bursaries from Sandoz and Novo Nordisk. P.B. reported consulting fees from Novo Nordisk, Ascendis and BioMarin. P.C. reported receiving consulting fees from Lumos and a research grant from Novo Nordisk. J.H.D. reported receiving consulting fees from BioMarin, KyowaKyrin and SANDOZ, speaker's fees from MedEA and a travel bursary from Novo Nordisk. T.Ed. reported receiving consultancy fees from Biomarin and Novo Nordisk. E.G reported receiving consultancy and speaker's fees and travel bursaries from Pfizer, Ascendis, Soleno, Radius Health, Novo Nordisk, Sandoz/Novartis and Kyowa Kirin. She is PI for clinical trials of Novo Nordisk, Soleno, AstraZeneca, Merck and Acadia. P.L. reported receiving consulting fees from Roche Institute and Pfizer. G.M. reported receiving consulting fees from Pfizer and Biomarin, participating on a data safety monitoring board from Kyowa Kirin and Sanofi, and serving

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Additional Information

Supplementary Information The online version contains supplementary available at xxxxxxxxxxxxxxxxxx

Box 1. Definitions

- In this guideline,
  - The word “child” is used for individuals between 0-<18 years.
  - “Genetic testing” refers to any form of DNA sequencing, copy number analysis or methylation analysis.
  - A “genetic cause” includes any chromosomal abnormality, (likely) pathogenic copy number variant (CNV), (likely) pathogenic DNA sequence variant, or methylation defect for which sufficient evidence exists to show a causal relationship with the individual's symptoms.
  - “Short stature (SS)” is used for all manifestations of growth failure, i.e., the presence of at least one out of three manifestations: a height below -2.0 SDS; a decreasing height SDS over time (growth faltering); and a height SDS below the lower limit of the statistically expected range around the sex-adjusted mid-parental height, expressed as the deviation from target height (TH) (height SDS-TH SDS <-1.5) or conditional target height (cTH) (height SDS-cTH SDS <-1.6).
  - “Short SGA” individuals are defined as born small for gestational age (SGA) with persistent SS.
  - “Chromosomal microarray (CMA)”, as used here, encompasses all types of array-based genomic copy number analyses, including array-based comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) arrays[99]. CNVs can also be detected by software programs in sequencing data.
- The “standard deviation score (SDS)” of an individual’s height is defined as the number of standard deviations above or below the mean for age and sex on a reference chart derived from the most recent respective population study.
- “Small for gestational age (SGA)” is defined as a reported birth weight and/or birth length below −2 SDS for gestational age.
- “Next-generation sequencing (NGS)” is a massively parallel sequencing technology that reads multiple DNA fragments in parallel with each other. Exome sequencing (ES) examines only exon sequences and intronic sequences nearby, i.e., only protein coding DNA sequences, that include approximately 2% of human DNA. Genome sequencing (GS) reads all the bases in DNA, i.e., includes exons, introns and non-coding intervening sequences. Both technologies can be used to examine single nucleotide variants (SNVs), small insertions and deletions, and copy number variations (CNVs) using different bioinformatic tools. However, some genomic rearrangements need to be confirmed using other methods (such as chromosome analysis, CMA, fluorescent in situ hybridization (FISH), or targeted sequencing of the breakpoints). Both technologies can be used to sequence only a patient’s DNA or in so-called family context when samples of biological parents or siblings can be used as reference samples for comparison. Currently, ES and GS use short reads. In a research setting, long read GS is available, a form of NGS that has technical advantages over short-read sequencing for the detection of specific types of genetic variation. It can sequence long strands of DNA or RNA without breaking them up into smaller fragments. Multiplex ligation-dependent probe amplification (MLPA) is a polymerase chain reaction (PCR) based method that uses probes to examine the copy number of a specific genomic region. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) is used, for example, in growth restricted imprinting disorders.

Box 2. Abbreviations used ≥2 times

- BA, bone age
- CDGP, constitutional delay of growth and puberty
- CMA, Chromosomal microarray (see definition)
- CNV, copy number variant
- ES, exome sequencing
- GH, growth hormone
- GHD, growth hormone deficiency
- GS, genome sequencing
- HSDS, height SDS
- IGF-1, insulin-like growth factor 1
- NGS, next-generation sequencing
- PCR, polymerase chain reaction
- QF-PCR, Quantitative Fluorescent Polymerase Chain Reaction
- rhGH, recombinant human growth hormone
- SDS, standard deviation score
- SGA, born small-for-gestational age
- SH/H, Sitting height/height ratio
- Short SGA, born small for gestational age (SGA) without catch-up growth
- SRS, Silver-Russell syndrome
- SS, Short stature (see definition)
- TH, target height

Box 3. Potential benefits and risks of genetic testing in children with SS

Potential benefits

- Definitive diagnosis can be gratifying to patients and their families
- Allows for more accurate genetic counseling and prediction of recurrence in other children
- Obviates the need for further extensive diagnostic tests to determine the etiology of the child's SS
- Enables earlier diagnosis by identifying a genetic condition before full phenotypic expression, particularly important in younger children
- Eliminates the need for GH stimulation tests
- Guides therapeutic decisions, e.g., deciding on prescribing growth stimulating medication
- Detects diagnoses for which rhGH is contraindicated or should be given with caution
- Highlights the need to screen for significant comorbidities associated with the underlying condition and refer to other specialties as needed
- Informs testing of additional family members allowing for earlier recognition of additional cases in the family
- Detects secondary genetic variants with potential to prevent adverse outcomes for the patient and their relatives

Potential risks

- A false positive genetic diagnosis leads to incorrect assumptions about the cause of disease, resulting in unnecessary anxiety, mismanagement, and inappropriate testing and treatment.
- Uncertainties arising from variants of uncertain significance reported.
- Secondary findings, even if accurate, can lead to anxiety in the affected family and, if erroneously classified, expose individuals to unnecessary surveillance or diagnostic testing.
- Secondary findings can affect certain types of insurance coverage. Laws vary by region.

Table 1: Characteristics of current molecular genetic techniques

Molecular genetic exam	Ability to identify (epi-)genetic variants						Limitations	
	SNVs and InDels	CNVs (resolution)	Repeat expansions	Inversions or translocation	Uniparental disomy	Aberrant methylation		Cost [g]
ANALYSIS APPROACH BASED ON CANDIDATE GENE/REGION								
FISH	-	+/- (500 kb [a])	-	+/-[e]	-	-	Only regions with commercial probes	$$
MLPA	-	+/- (< 1 kb [a])	-	-	-	-	Only regions with commercial kits	$$
MS-MLPA	-	+/- (< 1 kb[a])	-	-	Suggestive[e]	+	Only regions with commercial kits	$$
Single locus methylation test *	-	-	-	-	Suggestive[e]	+	No discrimination between aberrant methylation, UPD and CNV	$
Sanger sequencing	+	-	-	-	-	-		$
Panel NGS sequencing	+	+/- (< 1 kb[a])	-	-	-	-	Restricted number of genes	$$
DNA methylation episignatures	-	-	-	-	-	+	[f]	$$$$
HYPOTHESIS-FREE ANALYSIS (GENOMICS APPROACH)								
Karyotype	-	+/- (5-10 Mb)	-	+/-[d]	-	-	Requires cell culture and manual analysis by specialized cytogeneticist	$
CMA (SNP-array)	-	+ (50 kb[b])	-	-	+/-	-		$$
CMA (CGH-array)	-	+ (50 kb[b])	-	-	-	-		$$
Exome sequencing singleton	+	+/- (< 1 kb[c])	+/-[d]	+/-[d]	Suggestive[e]	-		$$
Exome sequencing trio/family	+	+/- (< 1 kb[c])	+/-[d]	+/-[d]	+	-		$$$
Genome sequencing - short reads - singleton	+	+ (< 1 kb)	+/-[d]	+/-[d]	Suggestive[e]	-	Limitations in evaluating variants in deep intergenic, regulatory and intron regions	$$$

Genome sequencing - short reads - trio/family	+	+ (< 1 kb)	+/-[d]	+/-[d]	+	-	Limitations in evaluating variants in deep intergenic, regulatory and intron regions	$$$$
Genome sequencing - long reads – singleton *	+	+ (< 1 kb)	+	+	Suggestive[e]	+	Requires DNA extraction technique preserving large intact fragments	$$$$
Genome Bisulfite Sequencing (GBS) *	+	+ (< 1 kb)	+/-[d]	+/-[d]	Suggestive[e]	+	Requires additional complex bioinformatic pipelines for conversion	NA
Optical Genome Mapping (OGM)	-	+ (< 1 kb)	+/-[d]	+	Suggestive[e]	-	Requires DNA extraction technique preserving large intact fragments of DNA	$$$

SNVs = Single nucleotide variant; InDels = Small insertions and deletions 1-50pb; CNVs = Copy number variants; FISH = Fluorescence In Situ Hybridization; MLPA = Multiplex Ligation-dependent Probe Amplification; MS-MLPA = Methylation-Specific MLPA; CMA = Chromosomal Microarray Analysis; CGH-array = Comparative Genomic Hybridization array; SNP-array = Single Nucleotide Polymorphism array; NGS = Next generation sequencing; UPD = Uniparental disomy

Single locus methylation test includes High Resolution Melting Analysis (HRMA); Methylation-Sensitive High Resolution Melting (MS-HRM) and Pyrosequencing

* Tests available in research environment only

\+ The test identifies the respective (epi-)genetic variants

+/- The test identifies the respective (epi-)genetic variants to a limited extent

\- The test does not identify the respective (epi-)genetic variants

a – Resolution of CNV detection limited to the analyzed regions

b – Smaller CNVs might be detectable by targeted analysis

c – Greater sensitivity when it affects 3 or more exons

d – Can identify, but with limitations

e – Suggestive result needs to be confirmed by another method

f – Only in leukocyte DNA; limited conditions defined by known signatures; does not identify causal genetic variant

g –Cost to the consumer. Comparison between the methods presented using $, $$, $$$ or $$$$. It is important to note that the availability and cost of genetic tests can vary significantly between countries, depending on local resources and healthcare systems.

Figure Legends

Figure 1. **Overview of the purpose and flow of the guideline**.

Figure 2. **Algorithm for the diagnostic work-up of children with short stature**. After a full clinical evaluation and exclusion of non-genetic secondary growth disorders, further diagnostic investigations depend on the clinical presentation, with the following categories: isolated short stature, skeletal dysplasia, defects in the GH/IGF axis, and syndromic short stature.

*In the (near) future, genome sequencing (short read or long read) will most likely become the standard approach in many countries making targeted panels obsolete. Most panels are currently performed *in silico*, i.e., genetic laboratories generate gene lists to analyse exome or genome sequencing data.

**Analysis of each case and the availability of resources should be considered in determining the best approach: exome sequencing (ES) or genome sequencing (GS); singleton, trio, or family analysis. In many cases, the use of ES incorporating CNV analysis can establish the diagnosis, but there is a growing application of genome sequencing (short and long read) which may become the preferred approach. The introduction of long-read genomic sequencing may also provide gene methylation information allowing for the diagnosis of short-stature disorders due to imprinting defects.

*** In selected cases, the first line of molecular analysis should be methylation assessment of specific regions related to an imprinting disorder.

Figure 1. Overview of the purpose and flow of the guideline.

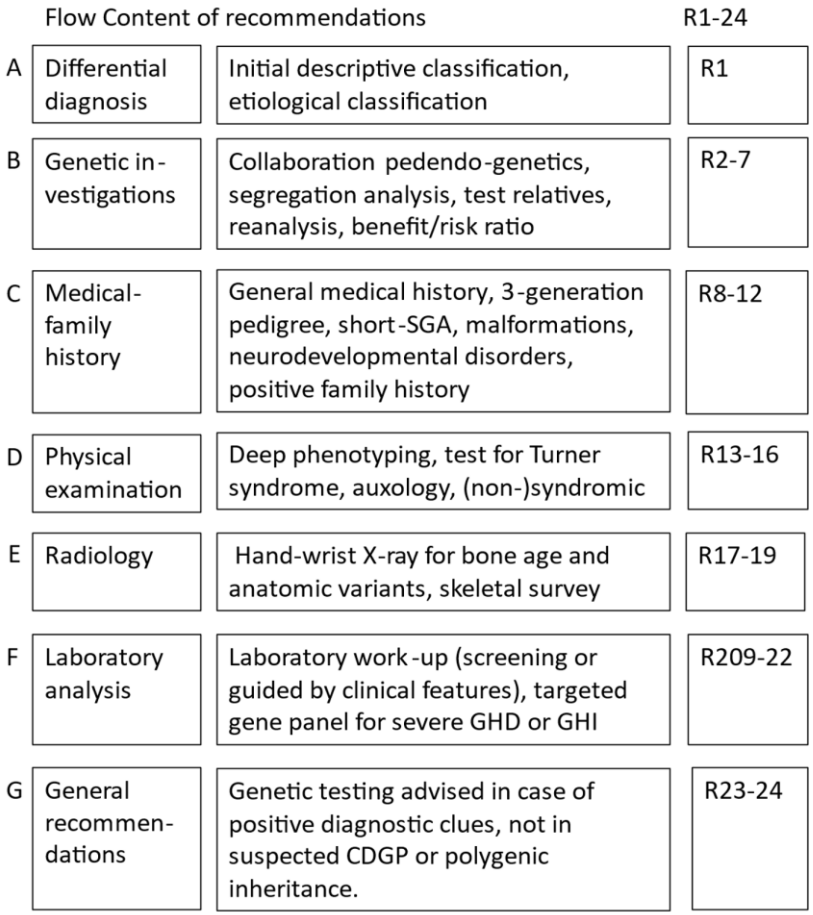

Figure 2 – Diagnostic Algorithm