

REVIEW

# Comprehensive genetic testing approaches as the basis for personalized management of growth disturbances: current status and perspectives

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## Abstract

The implementation of high-throughput and deep sequencing methods in routine genetic diagnostics has significantly improved the diagnostic yield in patient cohorts with growth disturbances and becomes increasingly important as the prerequisite of personalized medicine. They provide considerable chances to identify even rare and unexpected situations; nevertheless, we must be aware of their limitations. A simple genetic test in the beginning of a testing cascade might also help to identify the genetic cause of specific growth disorders. However, the clinical picture of genetically caused growth disturbance phenotypes can vary widely, and there is a broad clinical overlap between different growth disturbance disorders. As a consequence, the clinical diagnosis and therewith connected the decision on the appropriate genetic test is often a challenge. In fact, the clinician asking for genetic testing has to weigh different aspects in this decision process, including appropriateness (single gene test, stepwise procedure, comprehensive testing), turnaround time as the basis for rapid intervention, and economic considerations. Therefore, a frequent question in that context is 'what to test when'. In this review, we aim to review genetic testing strategies and their strengths and limitations and to raise awareness for the future implementation of interdisciplinary genome medicine in diagnoses, treatment, and counselling of growth disturbances.

## Key Words

- ▶ genetic testing
- ▶ growth disturbances
- ▶ growth retardation
- ▶ overgrowth
- ▶ genome medicine
- ▶ interdisciplinary clinical management

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## Introduction

Human growth is a complex physiological process, driven by the orchestrated expression of multiple genes and systems to control cell proliferation, cell hypertrophy, and extracellular matrix production, and be responsible for growth plate matrix production and growth plate chondrogenesis. It has a high degree of heritability based on common variants in genes implicated in different growth-associated pathways (1).

Growth disorders can be the result of non-genetic secondary factors, such as nutrition, chronic systemic disorders, and psychosocial deprivation, but in privileged countries, most forms of short and tall stature have probably an underlying primary genetic cause. They can be clinically divided into non-syndromic and syndromic disorders. In the case of syndromic disorders, complex symptoms in addition to growth disorders are observed. For a deeper understanding of the molecular mechanisms, the classification according to the underlying cause of a growth disorder is particularly important.

Multiple genetic anomalies are associated with short and tall stature and range from chromosomal variants to single bp changes and imprinting defects. Patients with large chromosomal variants (numerical chromosomal aberrations, (sub)microscopic copy number variants of several kb) most often have multiple comorbidities and associated features, since more than one gene is affected by the genetic alteration. Nevertheless, these syndromic growth disturbances can also occur in monogenetic disorders, depending on the physiological function of the disease-causing gene. Examples are the short stature KBG syndrome (OMIM 148050) based on *ANKRD11* variants (Table 1: case 1) and the Sotos overgrowth syndrome caused by *NSD1* variants. Altered growth can also be the only or major clinical sign, which is commonly associated with single gene variants. Variants in genes encoding members of the hypothalamo–pituitary axis were the first pathogenic changes which were identified to be responsible for growth retardation, but recently other monogenetic causes have also been considered as causative for idiopathic short stature (ISS), e.g. mutations in *SHOX* or *ACAN* (for reviews see 1, 2). Tall stature can also be caused by single gene variants like in *NPR2* or *NPR3* (3, 4, 5). However, the transition between syndromic and non-syndromic growth disturbances is not clearly defined: even in families with the same genetic variant, the phenotype can vary considerably, as is, for example, shown for *CDKN1C* variants in families with Beckwith–Wiedemann syndrome (BWS) (6). The clinical spectrum and severity

of phenotypic changes due to mutations within the same gene are influenced by their localization in the functional domains of the encoded protein, the type of the variant, the zygosity status, the imprinting status/the sex of the contributing parent, and modifying factors.

There is an increasing demand for precise molecular diagnosis as the basis for targeted clinical management, ranging from prognosis, monitoring of growth, the identification of disease-specific comorbidities, and the selection of the best treatment option. Advanced molecular technologies have exponentially increased our understanding of the underlying causes of short stature, but despite established clinical diagnostic and management recommendations, the decision on the molecular testing strategy in patients with growth disturbances can be a challenge in daily practice. In the past, molecular testing was hampered by limitations with respect to scope, availability, turnaround time, and costs. The continuing implementation of high-throughput next-generation sequencing (NGS) approaches (i.e. targeted NGS panels, whole exome sequencing (WES)) in routine genetic diagnostics of patients with growth disturbances is continuously increasing, which is reflected by the rising number of publications from 4 papers in 2011 to 76 in 2021 (<https://pubmed.ncbi.nlm.nih.gov/>, search terms ‘whole exome sequencing’, ‘diagnostic’, and ‘stature’). Accordingly, the diagnostic yield is permanently increasing (e.g. 7, 8, 9, 10, 11, 12, 13, 14), and NGS makes rapid diagnosis in acutely ill children with unclear diagnosis possible (15). The next step is the diagnostic use of whole genome sequencing (WGS), as it allows a more comprehensive view of our genome, and its feasibility has already been demonstrated (see below) (16, 17).

In everyday clinical practice, the question of the appropriateness of a genetic test continues to be addressed, and both the clinician and the geneticist must weigh the arguments for and against a genetic test based on the patient’s interest and the public health issues (‘when to test what’). In this review, we illustrate the power of current and future comprehensive molecular genetic strategies for an accurate and personalized diagnosis and assess their appropriate use.

## Clinical evaluation

Clinical evaluation of a child with a suspected growth disorder starts with a thorough past and current medical history including the family and social history, physical examination including anthropometric measurements,

**Table 1** Examples of real-live diagnostic odysseys from own experiences. It should be noted that these cases have been selected to illustrate possible diagnostic workups and stand for the experiences of labs which do not get precise clinical information.

Case (reference)	Case 1 (48, 65)	Case 2 (35)	Case 3 (66)	Unpublished
Clinical features	SGA, short stature, relative macrocephaly, prominent forehead, triangular face with pointed chin, prominent nose with high nasal bridge, large, dysplastic, and low-set ears, enlarged upper incisors. Normal psychomotoric development. (referred for SRS testing)	SGA, postnatal growth retardation, short stature, prominent forehead, feeding difficulties, body asymmetry, café-au-lait spots, Burkitt lymphoma at age 9 years. Normal psychomotoric development. (referred for SRS testing)	Prenatal and postnatal overgrowth, macroglossia, umbilical hernia, organomegaly, ear lobe creases, and occurrence of embryonal tumors as characteristic features. (referred for BWS testing)	SGA with persistent short stature, arthrogyposis multiplex congenita, facial dysmorphism (upslant, small, and low-set ears, short philtrum), oligodontia, ADHD, mild developmental delay. (combined phenotype of arthrogyposis and extremely short stature)
Conducted genetic tests	Array (referred for SRS testing)	MS-MLPA for 11p15.5, upd(7)mat and 14q32	MS-MLPA for 11p15.5	Karyotype, SNP array, MS-MLPA Chr7 and 11, WES (panel arthrogyposis)
Time to diagnosis	3 years	14 years	>20 years	11 years
Final test providing molecular diagnosis	SNP microarray	WES	Sanger sequencing	WES-MCA followed by trio-WES
Molecular result	Heterozygous de novo 348.4-kb deletion in 16q24.3 (incl. <i>ANKRD11</i> )	Homozygous <i>BLM</i> variant	Hemizygous <i>GPC3</i> variant	Heterozygous <i>PTPN11</i> and compound heterozygous <i>POLR3GL</i> variants
Final diagnosis	KBG syndrome	Bloom syndrome	Simpson-Golabi-Behmel syndrome	Noonan syndrome (NS)
Impact on counselling/therapy	AD inheritance; for management; see fact sheet: <a href="https://www.genetics.edu.au/PDF/ANKRD11_fact_sheet-CE.pdf">https://www.genetics.edu.au/PDF/ANKRD11_fact_sheet-CE.pdf</a>	AR inheritance, GH therapy contraindicated	XLR inheritance	AD inheritance (father and sister diagnosed after diagnosis in patient), GH therapy started at age 4 for indication SGA; dose change based on diagnosis NS. Screening for comorbidities associated with NS and <i>POLR3GL</i> variants.
Reason for odyssey	First classified as VUS, after recall and new database entries the deletion classified as pathogenic.	Spectrum of differential diagnosis of SRS was not overseen at first time of diagnosis.	Clinical overlap with BWS.	Dysmorphic features, severe short stature prior to start GH (-4 SDS) and clinical picture. Arthrogyposis not explained by most recent genetic findings.
Lessons learned	The variant was first classified as VUS, recall allows reclassification as pathogenic. Molecular karyotyping can reveal a diagnosis in patients with normal intellectual development.	GH treatment should not be recommended. The clinical spectrum of diseases evolves continuously.	Account for clinical overlap and non-specificity, discuss with clinical geneticist (not only) when a targeted test is negative.	Atypical or additional clinical features might be 'misleading': SGA and syndromic features area reason to continue search for underlying diagnosis.

AD, autosomal dominant; AR, autosomal recessive; BWS, Beckwith-Wiedemann syndrome; GH, growth hormone; MLPA, multiplex ligation-dependent probe amplification; MS-MLPA, methylation-specific MLPA; NS, Noonan syndrome; SGA, small for gestational age; SNP, single nucleotide polymorphism; SRS, Silver-Russell syndrome; VUS, variant of uncertain significance; WES, whole exome sequencing analysis; WES-MCA, WES-based molecular karyotyping; XLR, X-linked recessive.

and reconstruction of the child's growth curve. Once these data are analyzed and there is clinical suspicion of a growth disorder, the next steps include radiological and laboratory screening analyses to investigate secondary growth disorders and karyotyping or microarray analysis, e.g. in girls with growth failure to investigate Turner syndrome (Fig. 1). Numerous studies have published guidelines with stepwise diagnostic approaches to a child with a suspected growth disorder (10, 18, 19, 20, 21, 22). If radiologic and clinical examinations suggest an endocrine cause of the growth disturbance, growth hormone stimulation tests (GHSTs), serum insulin-like growth factor (IGF1), and sometimes an IGF1 generation test are used to investigate the growth hormone (GH)–IGF1 axis in more detail in patients with short stature (including in some cases IGF-BP3 and IGF-ALS measurements) and/or growth failure, and clinical and biochemical signs that raise suspicion for a GH–IGF1 axis disorder (23, 24). Interpretation of these endocrine test results remains challenging. Several publications have discussed cut-off IGF1 levels and cut-off peak GH levels during GHSTs to aid in predicting the likelihood of GH deficiency (GHD), the high rate of false positive tests in short children, and the lack of reproducibility of these tests (18, 19, 24, 25, 26). Children with short stature and no connective tissue defect likely have a continuum of GH–IGF1 axis defects (19, 27). Where the extreme forms of GHD and GH insensitivity are easily recognized, the milder forms are often challenging to diagnose especially in the light of the flaws/setbacks of endocrine testing.

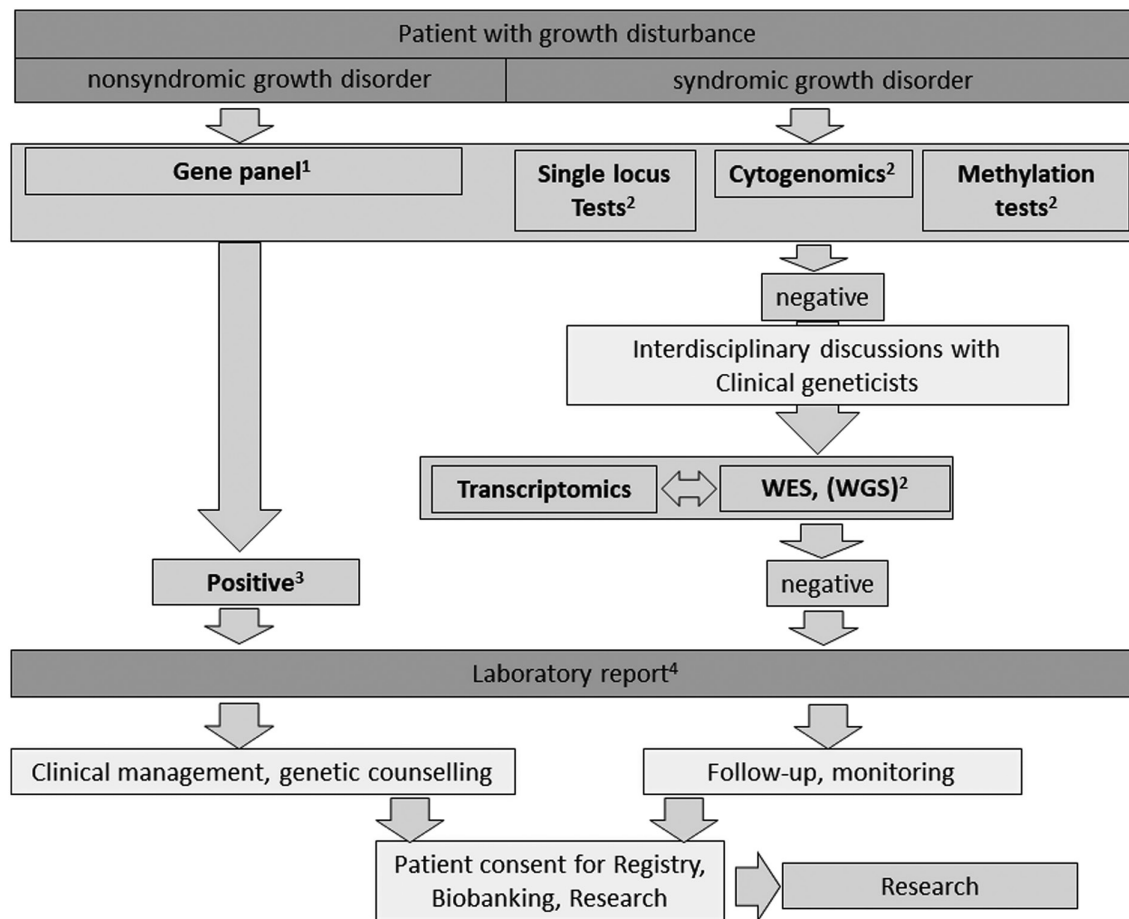
Part of the clinical examination is to describe the phenotype of a patient who presents with a growth disorder. Clinical scoring systems can guide physicians in their decision if genetic testing is indicated. Examples of clinical scoring systems are the Netchine-Harblison system for Silver–Russell syndrome (SRS) (28), the scoring system for *IGF1R* mutations (29), the revised Ghent criteria for Marfan syndrome (30), and a clinical score for Sotos syndrome (31). Features can sometimes be recognized as part of a well-known genetic syndrome. More often the disorder is not easily recognizable due to the rarity of the disorder, variability in phenotype, or only minor dysmorphic features (32). Advances in artificial intelligence and deep-learning algorithms have led to the development of tools and exchange platforms like Face2Gene (<https://www.face2gene.com>) and GestaltMatcher DB (<https://db.gestaltmatcher.org/>) as an aid to help clinicians to recognize syndromes based on facial morphologic features (33, 34) (Fig. 2).

## Currently applied molecular diagnostic methods

The benefit of genome medicine as the personalized therapeutic regime based on the genetic constitution of a patient leads to increasing demand for comprehensive genetic tests in pediatrics. Therefore, genetic tests help to refine a clinical diagnosis and to guide therapeutic approaches (Fig. 1), both in terms of the selection of the best treatment option and also in the decision of avoiding certain treatments (case 2: Bloom from 35). Furthermore, the molecular diagnosis is of significant value for genetic and reproductive counselling, as it contributes to precise information on the chance of recurrence in the family, and thereby supports the patient and his/her family in their self-determined process towards their decision on family planning (36, 37), including the option of prenatal testing.

Though we have entered the era of genome medicine with the chance to identify extremely rare molecular alterations in the whole genome, targeted assays and stepwise diagnostic algorithms should be considered as first-line tests in case of clinical features known to be associated with specific molecular alterations (Fig. 2 and Table 2).

Dependent on the spectrum of molecular changes in congenital disorders associated with single gene alterations, the appropriate methodology has to be chosen to ensure the reliable detection of the disease-causing variant: single nucleotide variants (SNVs) are commonly addressed by (Sanger) sequencing approaches, but this method fails to detect larger structural genomic variants, copy number variants (CNVs: deletions, duplications), or balanced rearrangements. The appropriateness of tests to target CNVs is influenced by the size of the expected genomic imbalance. Whereas single exon deletions or duplications can be analyzed in a routine genetic setting by multiplex ligation-dependent probe amplification (MLPA) or other techniques, larger CNVs are commonly addressed by cytogenomic techniques, i.e. microarray-based tests (SNP array or CGH array). However, balanced chromosomal rearrangements without any gain or loss of genetic material but with position effects are not detectable by these quantitative assays. Classically conventional chromosomal analysis and fluorescence in-situ hybridization (FISH) have been applied to encompass this group of mutations, but they are limited due to their low resolution of hundreds of kilobases (FISH) to megabases (chromosomal analysis). For genetic diagnostic testing of patients with growth disturbances, methylation at imprinted loci has to be



**Figure 1**

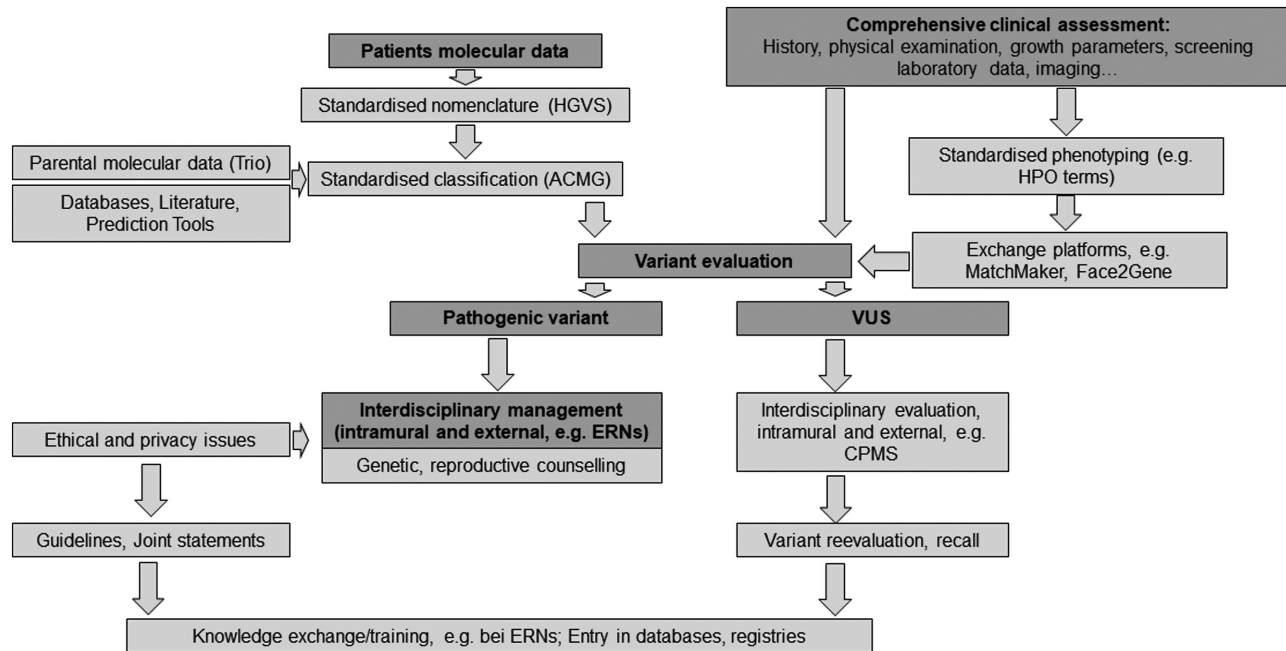
Diagnostic genetic testing algorithm for patients with growth disturbances. In case of non-syndromic disorders, specific molecular alterations addressed by single locus or methylation tests as well as chromosomal disturbances are not expected; therefore, a panel of genes involved in isolated growth might be suitable to be screened. <sup>1</sup>Gene panel: targeted analysis of specific genes, either based on a targeted enrichment or a targeted bioinformatics analysis of whole exome/genome data. The choice of genes might be leaned on <https://panelapp.genomicsengland.co.uk/panels>. <sup>2</sup>Decision on first-line test depends on the clinical phenotype of the patient, consensus guidelines, and on national regulations. For some disorders and phenotypes, targeted tests might be preferred; for some clinical indications (e.g. relatively non-specific growth disturbance, dysmorphisms, developmental delay), tests targeting the whole genome by cytogenomic tests or even WES or WGS might be suitable. Family history (more than one affected individual) should also be considered. <sup>3</sup>In case of a positive result of a first-line test, additional genetic tests might be required to confirm the first result, to characterize it more precisely, or to determine its inheritance as the basis for an adequate genetic counselling. <sup>4</sup>Depending on the disorder, national regulations, and clinical consensus guidelines, positive reports may also include recommendations for further action such as clinical management and counseling. In general, laboratory reports have to follow international standards (i.e. DIN ISO EN 15189).

addressed as aberrant imprinting marks significantly contribute to the etiology of imprinting disorders (e.g. SRS and BWS). Meanwhile, methylation-specific MLPA is widely accepted as a diagnostic tool for these molecular changes (Table 2).

The advantage of these single locus approaches is that they are often based on low-cost technologies, and the majority are fast and specific for a one or a small number of genetic loci or variants. However, this restriction to single genes and/or molecular variants is obviously the major limitation of single locus testing, in particular in the heterogeneous group of growth disturbances. In the

‘pre-NGS era’, patients with a presumed genetic diagnosis were, therefore, labor- and cost-intensive as well as time-consuming, in case the molecular cause was not identified at the beginning of the testing cascade. Many patients were left without a molecular diagnosis, with a long diagnostic odyssey accompanied by inappropriate clinical management and counseling (Table 1: cases 1, 3, 4).

With the development of NGS-based assays and their implementation in human genetic diagnostic settings, this major disadvantage of single locus testing is circumvented since a bundle of genes up to the whole genome can be analyzed in the same run now. In routine



**Figure 2**

Interdisciplinary and integrative decision strategy to improve diagnostics and management of patients with growth disorders. (American College of Medical Genetics (ACMG) criteria to standardize the pathogenicity of human genetic variants (44); Human Genome Variation Society (HGVS) recommends a standardized nomenclature of genetic variants (<https://varnomen.hgvs.org/>), variant of uncertain significance (VUS), human phenotype ontology (HPO; <https://hpo.jax.org/app/>), European reference network (ERN), and Clinical Patient Management System (CPMS) used by the ERNs).

diagnostics, different NGS-based diagnostic strategies have been validated and implemented. In fact, exome or even genome-wide approaches appear to be the most efficient and promising assays, the complementary use with long read assays or optical genome mapping will ensure a comprehensive identification of disease-causing molecular alterations.

Typical examples for single locus analysis by Sanger sequencing or methylation testing are Léri-Weill syndrome caused by *SHOX* mutations or BWS and SRS linked to imprinting defects on chromosome 11p15, respectively. On the other hand, the phenotypic outcome of these molecular changes is very broad, even contributing to non-syndromic growth retardation in case of *SHOX* (38) or rather unspecific phenotypes in case of 11p15 imprinting defects (39).

Thus today, clinicians can either order targeted single locus tests (e.g. *SHOX*), or cytogenomic tests to identify chromosomal alterations (see Table 2; e.g. in patients suspicious for Turner or Klinefelter syndrome) in case of patients with specific features. When these genetic tests come back negative, exome or genome-wide analyses can be conducted (WES, WGS) (Fig. 1).

A major challenge for all diagnostic tests is mosaicism, i.e. the occurrence of two or more genetically different

sets of cells. Mosaic mutations mostly originate from postzygotic errors after fertilization; therefore, they are not inherited. The severity of clinical symptoms depends on the time of the mutation event and can affect only specific tissues and sides of the body. Mosaicism, therefore, additionally contributes to clinical heterogeneity and is a diagnostic challenge requiring (ultra)deep targeted molecular analysis and testing of different tissues as shown for *PIK3CA*-associated overgrowth (40). In fact, the recent development of targeted therapies in the *PIK3CA* overgrowth illustrates the urgent need for precise molecular diagnosis and the power of precision medicine (41).

### Use of NGS-based techniques in routine diagnostics: chances and challenges

WES or (in the future) WGS testing should be carefully considered and ordered after consultation with clinical geneticists, as chances and challenges of uncertain or unexpected findings from genome-wide tests have to be considered in decision-making. On the other hand, the diagnostic value of WES/WGS is without question as it has contributed to a significant increase in diagnostic yield in a broad spectrum of pediatric disorders (see the

**Table 2** Overview on the application and limitations of the molecular methods commonly applied in routine diagnostic genetic testing, and future omic technologies which might be implemented in daily routine.

	SNVs <sup>a</sup> (scale)	CNVs <sup>a</sup> (scale)	Methylation testing	Balanced SVs <sup>a</sup>	Main applications/ advantages	Limitations	Examples (references)
<b>Single locus tests</b>							
Allele-specific PCR assays (e.g. ASO, ARMS)	Single bp	No	Possible	No	Low-cost screening for single SNVs	Only single and preselected SNVs are addressed in one tube	Rarely applied in growth diagnostics, rather used to identify a known variant in (affected) family members <i>SHOX</i>
Sanger sequencing	Up to 800 bp	No	Possible	No	Sequencing of small genes (e.g. <i>SHOX</i> )	High costs/bp, time-consuming	<i>SHOX</i> , <i>Beckwith-Wiedemann syndrome</i> (67)
<b>Methylation tests</b>							
MLPA	Possible	Up to 46 targets	Yes	No	CNV detection of specific genes, (multilocus) methylation analysis, low costs, fast	Restricted number of target loci	<i>Beckwith-Wiedemann syndrome</i> (68)
Pyrosequencing	40–60 bp	No	Yes	No	SNV and methylation of a small genomic stretch	Only small genomic regions can be addressed in one assay	
<b>Cytogenomics</b>							
Karyotyping	No	>5 Mb	No	>5 Mb	Detection of large structural variants and aneuploidies (e.g. Turner or Klinefelter syndrome)	Low resolution, time-consuming, sample preparation, subjective assessment	Turner syndrome, Klinefelter syndrome
FISH	No	100–200 kb	No	Possible	Second-line test to confirm suspected CNVs	Time-consuming, duplications might be difficult to be detected	Prader-Willi syndrome
Microarray (SNP, CGH)	No	>50 kb <sup>d</sup>	Possible (SNP array)	No	Detection of (sub) microscopic aberrations (e.g. syndromic patients)	Spatial rearrangements are not detected	Silver-Russell syndrome as second-line test (69)
Optical mapping	No	>500 bp	Research	Yes	High-resolution, spatial rearrangements are detectable	Sample preparation	(70)
<b>NGS assays</b>							
NGS panel (might be leaned on <a href="https://panelapp.genomicsengland.co.uk/panels">https://panelapp.genomicsengland.co.uk/panels</a> )	1.5–3 Mb <sup>b</sup>	Possible <sup>c</sup>	ImprintSeq	No	Suitable for heterogeneous disorders with specific clinical features, high coverage (mosaicism detectable), incidental findings rare	Only targeted loci are covered, untargeted variants escape detection	(40, 71)

(Continued)

**Table 2** Continued.

	SNVs <sup>a</sup> (scale)	CNVs <sup>a</sup> (scale)	Methylation testing	Balanced SVs <sup>a</sup>	Main applications/ advantages	Limitations	Examples (references)
Clinical exome	~4000 genes	Possible <sup>c</sup>	Research	No	All known disease-associated genes are addressed, suitable for disorders with unspecific clinical features	Increased probability for VUS and incidental findings. Fixed panel, new disease-associated genes not identifiable. Non-coding regions are not covered.	Suitable for prenatal testing to avoid incidental findings.
WES	1.1% of the total genome	Possible <sup>c</sup>	Research	No	All protein-coding regions are covered. Identification of new disease-causing genes possible. Suitable for disorders with unspecific phenotypes	Detection of VUS and incidental findings probable. Non-coding regions are not covered. Analysis and interpretation of large datasets required.	Heterogenous and unspecific phenotypes (72)
WGS	Whole genome	Possible <sup>c</sup>	Research	Possible <sup>e</sup>	Whole genome is analyzed, including non-coding regions. Suitable for disorders with unspecific phenotypes.	Detection of VUS and incidental findings highly probable. Processing, interpretation and storage of datasets required.	Heterogenous and unspecific phenotypes (17)
<b>Future omic technologies</b>							
Third-generation sequencing/long read sequencing (PacBio, Nanopore)	Whole genome	Possible	Research	Yes	Identification of all types of SVs, repeats, mosaic detection, determination of physical breakpoints.	General use in diagnostics in evaluation.	Application in progress
Transcriptomics	na	na	na	na	Identification of functional variants. Complementary tool for WES and WGS.	RNAs which are not expressed in the analyzed tissue are missed. Integration with data from other omic assays required	Application in progress

ARMS, amplification refractory mutation system; ASO, allele-specific oligonucleotide; na, not applicable; Possible, not commonly used in diagnostic context; SNVs<sup>a</sup>: 91%; substitutions, indel: 6%, deletion: 2%, insertion: 1% (according to Human variant class distribution – Ensembl 106); CNVs<sup>a</sup>: >50 bp; SVs<sup>a</sup>: inversions, translocations; <sup>b</sup>Recommended size to balance benefits with costs; <sup>c</sup>In case a bioinformatic analysis pipeline for CNV detection is implemented and validated; <sup>d</sup>The resolution of a microarray, rather than by the number of probes, is given by their spacing, i.e. the distance between the genomic position of a probe and the position of the next one.



'Introduction' section). Additional improvements can be expected by further progress and integration of methods, bioinformatics pipelines for lab data analyses, databases, and exchange platforms (Fig. 2).

The classification of the pathogenicity of a genetic variant is a major challenge nowadays, and the evaluation by clinical laboratory geneticists requires all information available from databases, literature, clinical data, and family history (Fig. 2). Standardization of the genetic and clinical classification is another important prerequisite to allow the exchange between clinicians and labs and to submit cases to public exchange platforms in order to further improve and enlarge clinical genetic databases as the basis for knowledge exchange, training, and future guidelines. In this context, the standardized coding of clinical diagnoses, e.g. as human phenotype ontology (HPO) terms, is becoming increasingly important for the basis for submission to databases and bioinformatic algorithm. All these activities significantly improve the diagnostic algorithms, even retrospectively by recall evaluation (Table 1: case 1) for the benefit of the patients and their families. Last but not least, these data compilations and exchanges have to consider ethical and privacy protection issues, in close collaboration with patient support groups and advocacies (e.g. in networks like ERNs).

Apart from financial considerations which might argue for a stepwise testing, clinicians ordering genetic tests as well as the laboratory geneticists themselves should keep in mind two further challenging unintended findings which can be obtained by NGS-based tests, i.e. variants of uncertain clinical significance (VUS) and incidental findings. The chance to detect these types of variants increases with the scale of genetic tests (Table 2), as impressively illustrated for genetic testing in hereditary breast cancer (42, 43).

Both VUS and incidental findings have the potential to create confusion for patients and their families, as well as for clinicians. VUS represent genetic variants for which the association with a disease is unclear at the time of their classification (44). In 2016, it has been estimated that up to 40% of genetic variants identified by NGS testing were classified as VUS (45), and the reason for the challenge to classify a variant as (likely) pathogenic or (likely) benign is that none or only scarce information is documented at the time of testing. A major task in translational medicine is therefore the documentation of all acquired information of a variant in public databases, and in combination with permanently improving bioinformatics and self-learning algorithms, the ratio of VUS among newly detected variants will decrease in the future.

There are contradictory arguments for and against reporting of VUS to clinicians and families: only VUS in disease-associated genes should be reported (46), and in diagnostic context, analysis should be focused on risk genes for the clinical diagnosis the test has been asked for. Except for some common genetic disorders, a periodic reevaluation of the pathogenicity of VUS is currently not available. Therefore, a recall after 2 or 3 years should be offered in the report (Fig. 1) (47), as the rationale for this procedure has been corroborated by numerous examples (e.g. (43), Table 1 – case 1 (48)).

The second challenge in NGS-based diagnostics is the identification and particularly the reporting of incidental findings (also called unsolicited findings), meaning pathogenic variants that are unrelated to the initial clinical questions but of clinical relevance. They differ from so-called 'secondary findings' which are pathogenic variants in genes not related to the original clinical diagnosis but actively looked for. As a growing number of genes is reported for which individuals carrying pathogenic variants remain without symptoms for several years, but preventive therapies exist, the American College of Medical Genetics has suggested a list of so-called 'medically actionable genes' (49). Numerous studies and suggestions have been published about the pros and cons of reporting incidental as well as secondary findings, and discussions are ongoing with variations in international policy documents (e.g. (50)). The healthcare providers involved in genetic testing should be aware of these discussions and the national rules and reporting practices. In particular, the information and permission of the patients and their families prior to diagnostic testing in respect to the handling of incidental findings must be considered. Therefore, addressing ethical and privacy issues is a major task in this context.

In addition to its suitability to analyze a huge number of disease-associated genes in parallel and its sensitivity to detect low-level mosaicism, a further advantage of WES and WGS techniques is the chance to identify rare, unexpected, and even new variants and genes. New monogenic growth disorders are constantly being diagnosed via both WES and WGS, whose disease value can also be proven because it is possible to bring together patients with rare and ultra-rare genetic disorders via exchange platforms such as Match-Maker and GeneMatcher. Thereby, these techniques as well as additional new methods (Table 2) have the potential to further increase detection rates as the basis of knowledge-driven personalized medicine. A prerequisite to reach the best benefit with *omic* result is their integrative analysis and interpretation, as well as submission to databases and registries (Fig. 2). With the

implementation of molecular and phenotype-driven exchange (self-learning) platforms in the processing of variant evaluation, the pathogenicity of variants may become more precisely and thus contribute to a better understanding of their pathoetiology and to a better personalized patient management.

## Personalized management from a clinician standpoint

The rapidly expanding possibilities of genetic testing challenge the clinician to choose the appropriate test in daily practice. After ruling out a secondary growth disorder, the clinician will have to decide what to do next. First, the decision must be made whether or not there is suspicion of a primary growth disorder. We should be aware to avoid medicalization of children who are not severely short or tall and do not have other clues that point toward a syndromic or monogenic disorder. If there are clues for a primary growth disorder or a genetic disorder associated with short or tall stature, a stepwise approach as highlighted in Fig. 1 can be of help.

Short stature after being born SGA and ISS are not clinical diagnoses, but definitions based on anthropometric data. The pretest likelihood of finding a genetic disorder in short SGA children is relatively high (approximately 30–40%), especially in case of additional features such as micro- or macrocephaly, dysmorphic features, developmental delay, and severe short stature ( $<-3$  SDS) (19). It is also important to consider genetic testing in these children to search for contra-indications for GH treatment, such as chromosomal breakage disorders (Table 1, case 3). A short child born SGA who develops elevated IGF1 levels during GH treatment, or who does not demonstrate catch-up growth, also warrants genetic investigation.

Genetic testing can guide therapeutic management. The indications for GH treatment have expanded, e.g. for short children due to *NPR2* and *ACAN* gene variants (51, 52, 53). However, also children with rare syndromes and severe short stature such as Wiedemann–Steiner syndrome, Okur–Chung syndrome, 18q deletion syndrome, and Aarskog syndrome can be offered a trial with GH treatment in various countries (54, 55, 56). Furthermore, genetic disorders such as *SHOX*, *ACAN*, *NPR2*, and *IGF1R* gene variants require higher doses of GH.

Sometimes a genetic diagnosis can identify potential future medical conditions that warrant follow-up and/or treatment, e.g. tumor predisposition in specific overgrowth syndromes.

Collaboration with or referral to a clinical geneticist or pediatric endocrinologist is advised. In specialized centers for rare growth disorders, one might benefit from a joint clinic of a pediatric endocrinologist and clinical geneticist (23). Thus, personalized medicine becomes a guidance for clinical evaluation, molecular evaluation, and therapy.

## Perspective I: future diagnostic strategies

Though the era of genome medicine has already started, future genome analysis needs further embedding in integrated and multidisciplinary approaches which consider all available laboratory and clinical data (Fig. 2). These ‘big data’ bear the potential for a personal and more precise medicine. The accumulation of these data may help to identify the progression from health to disease, helping to uncover preventable disease risk factors and allowing more precision diagnostic and prognostic information. Furthermore, it could be the basis for personalized treatment. At the population level, big data may help to integrate multiple social and environmental risk factors.

However, despite these enthusiastic future perspectives, the advantages and disadvantages of the new methods and their potential have to be carefully evaluated with respect to both individual and public health issues (57, 58).

As in the recent years has been shown, the power of NGS based assays lies in the chance to shorten and even avoid diagnostic odysseys (examples in Table 1), and thereby to allow a targeted clinical management as early as possible. Particularly, in acutely ill neonates and children, ‘rapid’ NGS approaches are promising prerequisites for customized acute therapeutic regimes and are in the process to be implemented in clinical settings (for review see 15).

## Perspective II: relevance for transition from child to adult care – long-term studies

Children with short stature as part of rare genetic syndromes often have multiple medical problems. Due to improved multidisciplinary care during childhood, life expectancy has increased, and transition to specialized adult care is needed for future care into adulthood. For many of the growth disorders, there is limited knowledge about their natural history, development of comorbidities, and best overall management. A multidisciplinary approach with a clinical algorithm was recently published (59). This can be

improved by centralized care, registries, or collaborations in networks such as the ERN-Endo.

Experiences with the application of recombinant human growth hormone (rhGH) for the treatment of GHD are available for almost 4 decades (60). The indications for GH treatment in non-GH-deficient children are expanding and often require higher doses of GH to induce catch-up growth, e.g. in short children due to *SHOX* gene variations.

Long-term safety of rhGH treatment has been investigated in GHD patients, short children born SGA, and patients with ISS (61, 62). No overall increased mortality risk was observed in patients without an underlying diagnosis that is associated with increased mortality (idiopathic GHD or ISS). In patients with an a priori increased risk of mortality due to the underlying diagnosis, no association was found between the increased risk of mortality and daily or cumulative GH dose which probably indicates there is no effect of GH treatment on mortality risk (61). GH treatment in children with short stature due to GHD, being born SGA, or ISS was associated with a small but increased risk of cardiovascular disease, especially in girls and short children born SGA (62). Being born SGA in itself is associated with an increased risk of cardiovascular disease (63).

Long-acting GH (LAGH) analogs are currently being marketed in the United States, Europe, and Asia for children and adults with GHD. Studies to investigate the efficacy and safety of LAGH for other indications are being executed. Possible safety concerns include metabolic effects related to the non-physiological profile of serum GH and IGF1 levels during treatment (64). Ongoing surveillance of patients who were treated with GH as a child is necessary since studies so far were performed in relatively young adults (<40 years of age). The international registry project GloBE-Reg ([www.GloBE-Reg.net](http://www.GloBE-Reg.net)) develops a newly developed platform to support long-term follow-up registry-based studies.

## Conclusions

The implementation of high-throughput and deep sequencing methods in routine genetic diagnostics has significantly improved the diagnostic yield in patient cohorts with growth disturbances and becomes increasingly important as the prerequisite of a personalized medicine. They provide considerable chances to identify even rare and unexpected situations; nevertheless, we have to be aware of their limitations and that simple genetic tests in the beginning of a testing cascade might also help

to identify the genetic cause of specific growth disorders. Existing and future networks like the ENDO-ERN will further improve the consenting on testing strategies as well as their standardization.

The clinician asking for genetic testing has to weigh manifold aspects in this decision process, including appropriateness (single gene test, stepwise procedure, comprehensive testing), turnaround time as the basis for a rapid intervention, and economic considerations. The future will see further increasing implementation of interdisciplinary genome medicine in diagnoses, treatment, and counselling of growth disturbances, with the maximum benefit for the patient and his family.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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### Author contribution statement

D K, A R and T E have drafted the paper. G B, I K, I N, G J, A H-K and M E have contributed specific aspects to the content, according to their field of expertise. All authors have approved the paper.

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