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Clinical relevance of systematic phenotyping and exome sequencing in patients with short stature

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Purpose: Short stature is a common condition of great concern to patients and their families. Mostly genetic in origin, the underlying cause often remains elusive due to clinical and genetic heterogeneity.

Methods: We systematically phenotyped 565 patients where common nongenetic causes of short stature were excluded, selected 200 representative patients for whole-exome sequencing, and analyzed the identified variants for pathogenicity and the affected genes regarding their functional relevance for growth.

Results: By standard targeted diagnostic and phenotype assessment, we identified a known disease cause in only 13.6% of the 565 patients. Whole-exome sequencing in 200 patients identified additional mutations in known short-stature genes in 16.5% of these patients who manifested only part of the symptomatology. In

15.5% of the 200 patients our findings were of significant clinical relevance. Heterozygous carriers of recessive skeletal dysplasia alleles represented 3.5% of the cases.

Conclusion: A combined approach of systematic phenotyping, targeted genetic testing, and whole-exome sequencing allows the identification of the underlying cause of short stature in at least 33% of cases, enabling physicians to improve diagnosis, treatment, and genetic counseling. Exome sequencing significantly increases the diagnostic yield and consequently care in patients with short stature.

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Key Words: growth; phenotypic spectrum; short stature; skeletal dysplasia; whole-exome sequencing

INTRODUCTION

Growth retardation, a common condition leading to reduced height, is defined as the deviation of an individual's height of more than two SDs below the mean in the population or the estimated familial target height.[1] The diagnosis is based on extent and type of growth retardation and other clinical signs and disorders.[1,2] Conditions with growth retardation include inborn errors of development, which also pose a risk of various additional health issues like cancer, stroke, and cardiac defects.[3–6] Along with these coexisting conditions, growth retardation might constitute a substantial emotional and clinical burden for affected individuals.

Short stature can be caused by nongenetic factors, such as nutrition, chronic systemic disorders, and emotional or psychosocial deprivation.[7] Most forms of short stature, however, are based on genetic causes. Turner syndrome,

SHOX defects, mutations in genes affecting the growth hormone signaling pathway, or rare skeletal dysplasias are well-known causes.[1,8–10] Nevertheless, the cause remains elusive in about 60–80% of patients, preventing early treatment of growth retardation and coexisting conditions as well as adequate genetic counseling.[11,12]

Human height is a polygenic trait with a heritability of about 80%.[13] Several genome-wide association studies (GWAS) have identified some 700 common variants explaining 20% of height variation in the normal population and recently an additional 1.7% have been shown to be caused by rare and low-frequency coding variants.[14,15] These studies probably explain some cases of short stature whereas recent studies suggest rare monogenic variants as the more common underlying cause.[16–19] In absence of a specific clinical phenotype, unbiased genome-wide approaches are necessary

to uncover such rare variants. We therefore investigated 565 patients with unexplained growth retardation by exhaustive clinical characterization followed by targeted genetic testing and exome sequencing in a representative subset of 200 patients.

MATERIALS AND METHDOS

Patients

We enrolled 565 consecutive patients and their families referred by local medical specialists for evaluation of growth retardation/short stature (**Table 1**, **Supplementary Figure S1** online). Of these, 551 patients were of European, 13 of Asian, and 1 of Arab descent. At time of enrollment, 83% of the patients were younger than 18 years. 81% presented with a height of 2 SDs below the age-related mean, whereas the remaining 19% were 2 SDs below the estimated target family height. Overall, 20% showed mild learning

Table 1 Clinical characteristics of included patients with idiopathic short stature

Characteristic	All 565 patients No. (%)	200 Exome patients[a] No. (%)
Age group		
< 4 y	102 (18)	33 (17)
> 4 y	463 (82)	167 (83)
Small for gestational age	156 (28)	59 (30)
Short stature (SDs)		
−2 to −3	270 (48)	99 (50)
−3 to −4	133 (24)	48 (24)
−4 to −5	34 (6)	17 (8)
< −5	19 (3)	9 (4)
Below est. height	109 (19)	27 (14)
Short stature type		
Isolated	384 (68)	134 (67)
Syndromic	181 (32)	66 (33)
Head circumference (SDs)		
> −2	448 (79)	140 (70)
−2 to −3	60 (11)	26 (13)
−3 to −5	45 (8)	27 (14)
< −5	12 (2)	7 (3)
IQ		
Normal	450 (80)	160 (80)
70–85	115 (20)	40 (20)
Sex		
Female	349 (62)	122 (61)
Male	216 (38)	78 (39)
Bone age		
Accelerated	16 (3)	11 (5)
Normal	27 (5)	19 (10)
Delayed	125 (22)	68 (34)
Not available	397 (70)	102 (51)

[a]200 representative patients from the 565 where the prior targeted clinical and diagnostic approach had not led to the identification of an underlying cause. These patients showed no statistical difference for each single or combined characteristic.

disabilities and 21% microcephaly. 30% underwent bone age evaluation and of those 84% had either delayed or accelerated bone ages. All 565 patients underwent extensive prior endocrinological and diagnostic workup to exclude defects of the growth hormone pathway and organic causes of their growth deficit. All procedures were in accordance with the ethical standards of the FAU Erlangen-Nürnberg and the Helsinki Declaration. Detailed recruitment information is provided in the Methods section in the **Supplementary Data**.

Systematic phenotyping and targeted testing

On enrollment, all 565 patients received extensive genetic evaluation including syndromic and radiographic assessment by a clinical geneticist according to a standardized questionnaire. All information was included in a database based on known phenotype terms, followed by assessment of published information and discussion with a review board of experts in clinical genetics and dysmorphology (systematic phenotyping, Methods section in the **Supplementary Data**).[20] Targeted genetic testing was applied based on known disease frequencies and phenotypic characteristics.

Exome sequencing and variant assessment

After exclusion of individuals from the group of 565 patients in whom our genetic targeted clinical and diagnostic approach had led to the identification of an underlying genetic cause of growth retardation, we selected a representative group of 200 patients (**Figure 1**, **Table 1** and **Supplementary Figure S1**). These 200 patients showed no statistically significant difference from the remaining group of patients regarding age, height distribution, stature type, development, bone age, and sex (**Table 1** and **Supplementary Figure S1**; selection and clinical description of the 200 individuals for exome analysis is provided in the Methods section in the **Supplementary Data**).

We performed whole-exome sequencing in this group of 200 patients—100 patients and both parents (trio analysis) and 100 patients (affected-only analysis)—after enrichment by SureSelect targeted capturing (**Figure 1**, **Supplementary Figures S1–S2**). Exomes were analyzed with our custom NGS Variant Analyzer tool, which involves the semiautomatic selection and data quality inspection of variants followed by the interpretation in relation to the reported phenotypic spectrum. The veracity and segregation in the families of selected variants were confirmed using Sanger sequencing.

All 200 patients were analyzed with respect to 1,000 known growth related genes derived from OMIM and MedGen databases (**Supplementary Table S2**). We considered causal all variants in known short stature–associated disease genes when the variant was predicted pathogenic or likely pathogenic referring to American College of Medical Genetics and Genomics (ACMG) criteria[21] and segregating with the phenotype in the family (**Supplementary Data**). All results were followed by a genotype–phenotype reevaluation. Detailed information about exome sequencing
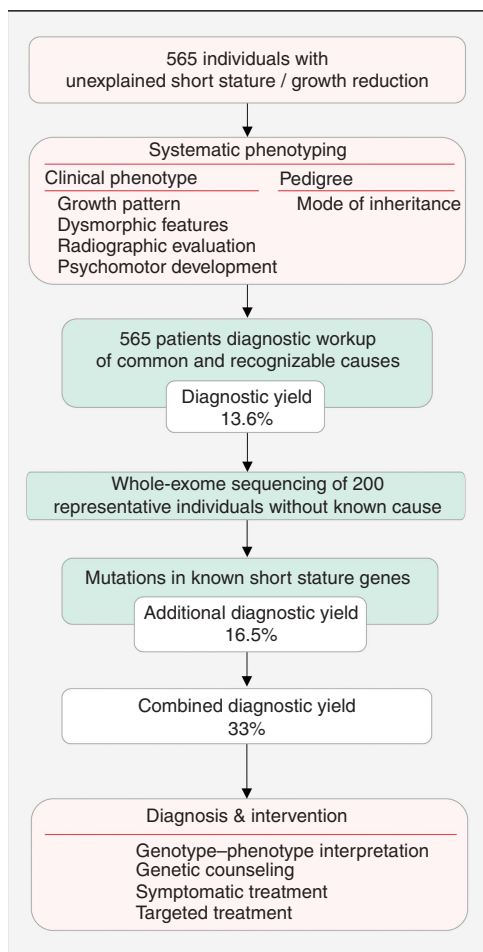
Figure 1 Flowchart gene discovery approach. We built a study group of 565 individuals with short stature or growth retardation (**Table 1**). Systematic phenotyping and targeted diagnostic testing of common and recognizable causes in all 565 patients led to a diagnostic yield of 13.6% (**Supplementary Table 1**). Detailed information about the systematic phenotyping is provided in the Methods section in the **Supplementary Data**. For 200 representative individuals where no underlying cause could be determined (**Table 1**), we performed whole-exome sequencing, which increased the diagnostic yield by 17% up to 33% by the identification of mutations in known short stature–associated genes (**Table 2**, **Supplementary Tables 3–4**). Genotype–phenotype re-evaluation confirmed that these patients present with only part of the characteristic symptomatology. The additional diagnostic yield already had direct effects in preventive measures, symptomatic and targeted treatment in 15.5% of the 200 exome-sequenced patients (**Table 4** and **Supplementary Table S6**).

and interpretation of variants in known short-stature genes is provided in the Methods section in the **Supplementary Data**.

Data availability
Data for various analyses have been mentioned throughout the text and derived data supporting the findings of this study are available from the corresponding author upon request. Any other data associated with this study are available in the **Supplementary Data**. Additional methods are described in the **Supplementary Data**.

RESULTS
Diagnostic yield of common and recognizable phenotypes in 565 individuals
We could establish a diagnosis after systematic phenotyping in 13.6% of patients including disorders due to variants in 10 genes, imprinting defects, large chromosomal aberrations, and 24 distinct copy-number variants (**Supplementary Table 1**). Copy-number variants ranging in size from 0.1 to 14.2 Mb were the most common cause of nonspecific syndromic short stature, identified in 6.9% of the patients. A syndromic form of short stature was suspected in 181 patients and confirmed in 26 of them (4.6% of the 565 patients), mostly *SHOX*-related short stature and Silver–Russell syndrome. Turner syndrome or Turner syndrome variants were found in 1.6% of the patients and mutations in genes associated with skeletal dysplasia in 0.5%. No diagnosis could be established in 491 (86.4%) of the patients.

Diagnostic yield of exome sequencing in 200 individuals
To confirm the power of family-based exome sequencing in providing a diagnosis in individuals with growth retardation we selected a representative group of 200 families of the remaining patients with unexplained growth retardation (**Figure 1**, **Table 1**, and **Supplementary Figure 1**). We considered genes associated with growth phenotypes according to OMIM and MedGen databases and in these confirmed variants with regard to segregation and the specific patient's phenotype. Thereby, we identified 38 variants affecting 26 genes in 33 of 200 exome patients (16.5% of the exome group; **Table 2**, **Supplementary Figures S3–S27** and **Supplementary Tables S2–S4**). Of the 38 variants, 27 (71%) were missense, 5 (13%) frameshift, 4 (11%) nonsense, and 2 (5%) canonical splice-site variants. Twenty-nine of the 38 variants were not reported in the Exome Aggregation Consortium database, and nine were rare, with a maximum frequency of 2×10^{-4}. Following the ACMG guidelines,[21] 16 of the identified 38 variants (42%) were classified as likely pathogenic and the remaining 22 (58%) were defined as pathogenic variants (Materials and Methods section, **Table 2**, and **Supplementary Table S4**). The median height of these 33 patients was 2.7 SDs below the average height in the population, 21% showed microcephaly, and 41% were classified as syndromic (**Supplementary Figure S1** and **Supplementary Table S3**). Modes of inheritance were autosomal dominant (65%), autosomal recessive (19%), and X-linked recessive (15%) (**Table 2**, **Supplementary Tables S3–S4**). Pathway analyses of the affected proteins revealed that 58% of them are involved in the main functional categories of cartilage formation, chromatin modification, and Ras-MAPK signaling (**Table 3**).

We further wanted to know whether the selection of specific phenotypes or a combination of phenotypic features might reveal a higher diagnostic yield. A support vector machine approach resulted in 85.5% probability that it is not currently

Table 2 Genetic diagnosis obtained by exome sequencing in 200 individuals with idiopathic short stature

Patient	Gene	Gender	Inheritance	Genomic mutation (HGVS)	cDNA	Protein	ACMG category	Diagnosis
Trio 28	ACAN	F	Maternally inherited	chr15(GRCh37):g.89388864C > T	NM_013227.3: c.1180C > T	p.(Arg394*)	Pathogenic (Ib)	Osteochondritis dissecans with short stature
AffOnly 26	ACAN	F	Maternally inherited	chr15(GRCh37):g.89383303del	NM_013227.3:c.515del	p.(Gln172Argfs*59)	Pathogenic (Ib)	Osteochondritis dissecans with short stature
AffOnly 47	ACAN	M	Maternally inherited	chr15(GRCh37):g.89392710C > T	NM_013227.3: c.1774C > T	p.(Gln592*)	Pathogenic (Ib)	Osteochondritis dissecans with short stature
AffOnly 62	ACAN	M	De novo	chr15(GRCh37):g.89401413C > A	NM_013227.3: c.5597C > A	p.(Ser1866*)	Pathogenic (Ia)	Osteochondritis dissecans with short stature
AffOnly 89	ACAN	F	Paternally inherited	chr15(GRCh37):g.89381974T > G	NM_013227.3:c.151T > G	p.(Cys51Gly)	Likely pathogenic (V)	Osteochondritis dissecans with short stature
Trio 11	ANKRD11	M	De novo	chr16(GRCh37): g.89351174_89351180del	NM_001256182.1: c.1770_1776del	p.(Pro591Glyfs*60)	Pathogenic (Ia)	KBG syndrome
Trio 58	CASK	M	Hemizygous	chrX(GRCh37):g.41485893C > T	NM_003688.3: c.979G > A	p.(Glu327Lys)	Likely pathogenic (V)	FG syndrome
Trio 67	CLCN5	M	Hemizygous	chrX(GRCh37):g.49834668C > T	NM_001127899.1: c.298C > T	p.(Arg100Trp)	Likely pathogenic (V)	Hypophosphatemic rickets
Trio 38	COL2A1	M	Paternally inherited	chr12(GRCh37):g.48383569C > A	NM_001844.4: c.1043G > T	p.(Gly348Val)	Likely pathogenic (V)	Stickler syndrome
Trio 62	COL2A1	M	De novo	chr12(GRCh37):g.48370611C > G	NM_001844.4: c.3419G > C	p.(Gly1140Ala)	Pathogenic (IIIb)	Stickler syndrome
AffOnly 4	CUL7	M	Homozygous	chr6(GRCh37):g.43011369C > G	NM_001168370.1: c.3425-1G > C	p.?	Pathogenic (Ib)	3-M syndrome
Trio 27	FGD1	M	Hemizygous	chrX(GRCh37):g.54491974G > C	NM_004463.2: c.1546C > G	p.(Pro516Ala)	Likely pathogenic (V)	Aarskog syndrome
AffOnly 97	FGFR3	M	Maternally inherited	chr4(GRCh37):g.1807363A > G	NM_000142.4: c.1612A > G	p.(Ile538Val)	Pathogenic (IIIb)	Hypochondroplasia
AffOnly 95	FLNB	F	Compound heterozygous	chr3(GRCh37):g.[58149042G > A]/ [58090836C > T]	NM_001164317.1: [c.7276G > A]/ [c.1640C > T]	[p.(Glu2426Lys)]/ [p.(Ala547Val)]	Likely pathogenic (V) / Likely pathogenic (V)	Spondylocarpotarsal synostosis syndrome
Trio 18	GHSR	F	Maternally inherited	chr3(GRCh37):g.172163003G > C	NM_198407.1: c.1049C > G	p.(Thr350Ser)	Likely pathogenic (V)	Isolated partial growth hormone deficiency
AffOnly 77	HDAC6	M	Hemizygous	chrX(GRCh37):g.48681063A > G	NM_006044.2: c.2371A > G	p.(Met791Val)	Likely pathogenic (V)	Chondrodysplasia with platyspondyly
AffOnly 37	IFT140	M	Compound heterozygous	chr16(GRCh37):g.[1573854T > A]/ [1642549C > T]	NM_014714.3: [c.3245A > T]/ [c.410G > A]	[p.(Asp1082Val)]/ [p.(Arg137Gln)]	Likely pathogenic (IV)/ Likely pathogenic (V)	Mainzer–Saldino syndrome
AffOnly 65	IGF1R	M	Maternally inherited	chr15(GRCh37):g.99500379A > G	NM_000875.3: c.3812A > G	p.(Glu1271Gly)	Likely pathogenic (V)	Resistance to insulin-like growth factor 1
AffOnly 84	IHH	M	Maternally inherited	chr2(GRCh37):g.219920354G > A	NM_002181.3:c.811C > T	p.(Leu271Phe)	Likely pathogenic (V)	Brachydactyly, type A1

Table 2 Continued

Patient	Gene	Gender	Inheritance	Genomic mutation (HGVS)	cDNA	Protein	ACMG category	Diagnosis
AffOnly 68	KAT6B	M	De novo	chr10(GRCh37):g.76790228del	NM_012330.3:c.5646del	p.(Asn1883Thrfs*2)	Pathogenic (Ia)	Genitopatellar syndrome
Trio 2	KDM6A	F	De novo	chrX(GRCh37):g.44922973C > T	NM_021140.2:c.1834C > T	p.(Arg612*)	Pathogenic (Ia)	Kabuki syndrome 2
AffOnly 96	KDM6A	F	De novo	chrX(GRCh37):g.44894175G > A	NM_021140.2:c.565-1G > A	p.?	Pathogenic (Ia)	Kabuki syndrome 2
Trio 10	KRAS	M	De novo	chr12(GRCh37):g.25362838T > C	NM_004985.3:c.458A > G	p.(Asp153Gly)	Pathogenic (II)	Noonan syndrome spectrum
Trio 5	MAP2K1	M	De novo	chr15(GRCh37):g.66729175G > C	NM_002755.3:c.383G > C	p.(Gly128Ala)	Pathogenic (IIIa)	Noonan syndrome spectrum
AffOnly 44	MATN3	M	De novo	chr2(GRCh37):g.20194143G > A	NM_002381.4:c.1322C > T	p.(Ser441Phe)	Pathogenic (IIIb)	Multiple epiphyseal dysplasia
AffOnly 50	NF1	F	De novo	chr17(GRCh37):g.29554304A > C	NM_001042492.2:c.2320A > C	p.(Thr774Pro)	Pathogenic (IIIb)	Neurofibromatosis type 1
Trio 28	NPR2	F	Paternally inherited	chr9(GRCh37):g.35799682T > A	NM_003995.3:c.941T > A	p.(Leu314Gln)	Likely pathogenic (V)	Short stature with nonspecific skeletal abnormalities
AffOnly 17	NPR2	F	Paternally inherited	chr9(GRCh37):g.35808587C > T	NM_003995.3:c.2794C > T	p.(Arg932Cys)	Likely pathogenic (V)	Short stature with nonspecific skeletal abnormalities
AffOnly 85	NPR2	F	De novo	chr9(GRCh37):g.35802239C > T	NM_003995.3:c.1669C > T	p.(Arg557Cys)	Pathogenic (IIIb)	Short stature with nonspecific skeletal abnormalities
Trio 77	PDE3A	F	De novo	chr12(GRCh37):g.20769240G > A	NM_000921.4:c.1346G > A	p.(Gly449Asp)	Pathogenic (IIIa)	Hypertension and brachydactyly syndrome
AffOnly 72	PDE4D	F	De novo	chr5(GRCh37):g.58334711G > T	NM_001104631.1:c.896C > A	p.(Ser299Tyr)	Pathogenic (IIIb)	Acrodysostosis 2
AffOnly 74	PTPN11	M	De novo	chr12(GRCh37):g.112915523A > G	NM_002834.3:c.922A > G	p.(Asn308Asp)	Pathogenic (II)	Noonan syndrome spectrum
AffOnly 23	SLC26A2	M	Compound heterozygous	chr5(GRCh37):g.[149361113T > A]/[149357568T > A]	NM_000112.3:[c.1957T > A]/[c.353T > A]	[p.(Cys653Ser)]/[p.(Val118Glu)]	Pathogenic (IIIa)/Likely pathogenic (IV)	Multiple epiphyseal dysplasia 4
AffOnly 57	TRIM37	M	Compound heterozygous	chr17(GRCh37):g.[57093086dupl/57094665_57094666del]	NM_001005207.2:[c.2461dup]/[c.2377_2378del]	[p.(Ile821Asnfs*6)]/[p.(Leu793Valfs*2)]	Pathogenic (Ib)/Pathogenic (Ib)	Mulibrey nanism

ACMG, American College of Medical Genetics and Genomics; AffOnly, affected only; cDNA, complementary DNA; F, female; HGVS, Human Genome Variation Society; M, male.

Table 3 Diagnostic yield of exome sequencing in 200 patients

Main functional category[a]	No. of genes	Identified genes (no. of patients affected)
Cartilage formation	6	*ACAN* (5), *COL2A1* (2), *FGFR3* (1), *IHH* (1), *MATN3* (1), *SLC26A2* (1)
Chromatin modification	5	*KDM6A* (2), *ANKRD11* (1), *HDAC6* (1), *KAT6B* (1), *TRIM37* (1)
Ras-MAPK pathway	4	*KRAS* (1), *MAP2K1* (1), *NF1* (1), *PTPN11* (1)
Growth hormone–related pathway	2	*GHSR* (1), *IGF1R* (1)
Regulation of cytoskeleton	2	*FGD1* (1), *FLNB* (1)
cAMP signaling pathway	2	*PDE3A* (1), *PDE4D* (1)
Centrosome/cilia formation	2	*CUL7* (1), *IFT140* (1)
mTOR signaling pathway	1	*NPR2* (3)
Transcription regulation	1	*CASK* (1)
Renal regulation	1	*CLCN5* (1)

cAMP, cyclic adenosine monophosphate; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin.
[a]From DAVID functional annotation, OMIM, and KEGG.

possible to accurately predict the presence of a pathogenic mutation based on one or a combination of clinical subgroups (height, occipitofrontal circumference, intellectual disability, syndromic versus isolated phenotype, prenatal growth retardation, and accelerated/decelerated bone age) (Methods section in the **Supplementary Data**).

Overall, considering only genes previously associated with growth retardation, the diagnostic yield of 13.6% achieved with systematic phenotyping and targeted testing was raised to 33% by additional exome sequencing (**Figure 1**). The most commonly mutated known short stature–associated genes identified were *ACAN* (2.5%), *NPR2* (1.5%), *KDM6A* (1%), and *COL2A1* (1%) (**Table 2** and **Supplementary Tables S3–S4**).

Expansion of the phenotypic spectrum by exome sequencing

Some of the mutations identified by exome sequencing are located in genes known to be associated with syndromic intellectual disability or skeletal dysplasia (**Table 2** and **Supplementary Tables S3–S5**). One patient with a novel pathogenic *KRAS* missense mutation presented with proportionate short stature and learning disability illustrating the mild end of the Noonan syndrome spectrum disorders.[22] Two patients with *KDM6A* mutations demonstrated the phenotypic variability of Kabuki syndrome.[23,24] Neither showed any signs of developmental delay but they presented with part of the characteristic facial gestalt. Short stature is also an essential symptom of many skeletal dysplasias.[9] We found mutations in *FGFR3*, *COL2A1*, and *SLC26A2* in four patients presenting with no obvious specific skeletal involvement at initial clinical evaluation but consistent with the mild end of the spectrum of these entities upon re-evaluation. Finally, we identified a previously unreported likely pathogenic hemizygous missense mutation in the calcium/calmodulin-dependent serine protein kinase gene (*CASK*) in a male patient with mild short stature and microcephaly but no intellectual disability. Loss-of-function *CASK* mutations underlie several forms of X-linked intellectual

disability syndromes and patients are reported to present with short stature.[25]

Carriers of recessive skeletal dysplasia mutations present with idiopathic short stature

In 3.5% of the 200 exome patients we identified heterozygous mutations in two genes (*ACAN*, *NPR2*) previously reported to cause the autosomal-recessive skeletal dysplasias spondyloe-pimetaphyseal dysplasia and acromesomelic dysplasia, respectively (**Table 2**, **Supplementary Tables S3–S5**).[9,26] Heterozygous carriers consistently show idiopathic short stature without dysmorphic findings.[26–30] We found heterozygous mutations in *ACAN* in five and *NPR2* in three patients who were not previously suspected to be carriers. The height of these patients ranged between − 2.0 and − 4.7 SDs (**Supplementary Table S3**). One patient carried both a variant in *NPR2* and a variant in *ACAN*. This could indicate a blended phenotype as previously reported.[31]

Additional relevance of exome sequencing results for clinical management

Besides the increased diagnostic yield by exome sequencing in the 200 patients with idiopathic short stature, results with possible impact on treatment or additional preventive measurements occurred in 31 families (15.5% of 200 exome individuals, **Table 4** and **Supplementary Table S6**). This led to preventive measures for osteoarthritis (*ACAN*, *CUL7*, *MATN3*) and neoplasias (*NF1*, *PTPN11*, *TRIM37*), as well as orthopedic support and regular developmental evaluation in affected individuals from 23 families (11.5%). Symptomatic treatment or screening for associated malformations (*KAT6B*, *KRAS*, *MAP2K1*, *PTPN11*), hearing loss (*COL2A1*, *FLNB*), or the risk for chronic kidney disease (*IFT140*, *CLCN5*) was recommended in nine families (4.5%). A treatment with recombinant growth hormone or IGF1 has been shown to be beneficial especially in the individuals with defects of the growth hormone pathway.[32–34] We identified mutations affecting genes of this pathway (*GHSR*, *IGF1R*) allowing specific treatment in three families (1.5%). Interestingly, these have been missed by endocrine testing, but were confirmed by

Table 4 Intervention in affected 200 exome individuals with mutations in known short-stature genes

Type	Symptom	Genes[a]
Preventive measures	Osteoarthritis	ACAN, CUL7, MATN3
	Hearing loss	COL2A1
	Orthopedic symptoms	COL2A1, FGFR3, IHH, SLC26A2, HDAC6, FLNB
	Developmental issues	KDM6A, ANKRD11, PDE4D, CASK, FGD1, PTPN11, NF1
	Bleeding diathesis	PTPN11
	Neoplasia	TRIM37, PTPN11, NF1
Symptomatic treatment	Hearing loss	COL2A1, FLNB
	Multiple malformations	KAT6B, KRAS, MAP2K1, PTPN11
	Chronic kidney disease	IFT140, CLCN5
Targeted treatment	Growth hormone signaling pathway defects	GHSR, IGF1R, PTPN11
	Severe hypertension	PDE3A

[a]Information derived from *GeneReviews* and other publications (**Supplementary Table S5**).

growth hormone stimulation tests and therefore considered at least to contribute in part to the patient's growth phenotype. Also immediately relevant was the identification of a *PDE3A* mutation in one girl. Activating mutations in *PDE3A* led to hypertension and brachydactyly syndrome (MIM 123805) with the development of life-threatening hypertension later in life.[35] A targeted treatment with phosphodiesterase inhibitors is indicated due to its potentially significant impact on life expectancy.[36] Finally, knowledge about the inheritance pattern will aid evaluation of the recurrence risk and improve genetic counseling of families.

DISCUSSION

Even though the heritability of short stature is considered high, the large number of genes implicated and the nonspecific clinical phenotype have led to poor yield of diagnostic genetic testing.[13,16,18,19,37–39] To demonstrate the diagnostic outcome we enrolled 565 carefully characterized patients with short stature and their families and established an interdisciplinary systematic phenotyping with additional targeted gene testing (**Figure 1**). This led to a diagnostic yield of 13.6% including copy-number variants, chromosomal aberrations, and monogenic causes (**Supplementary Table S1**). We next performed unbiased exome sequencing in 200 representative patients with short stature of unknown origin (**Table 1** and **Supplementary Figure S1**). The variant evaluation and classification in accordance with the ACMG guidlines[21] led to the identification of mutations in known short stature–associated genes in 33 affected individuals (16.5%) (**Table 2**, **Supplementary Tables S3–S5**). In a previous smaller study of 14 highly selected patients a diagnostic yield of 36% was reported,[16] which might be explained by the study's strict inclusion criteria and smaller sample size. Genotype–phenotype re-evaluation confirmed that the individuals from our exome study were lacking most of the characteristic features of the entities, indicating that current descriptions are biased and that the phenotypic spectrum needs to be expanded. This is especially true

for genes associated with syndromic forms of short stature and intellectual disability as well as skeletal dysplasias. There are parallels to the field of intellectual disability, also plagued by high genetic heterogeneity and unspecific clinical presentation, where both a similar mutation yield and inheritance modes were reported.[40] However, in this study clinical subgrouping did not reveal any correlation between a specific phenotypic feature or combination of them and the probability to identify a mutation, thus supporting an unbiased approach such as exome analysis for all patients with idiopathic short stature.

One remarkable aspect was the detected frequency of heterozygous mutations in genes previously implicated in autosomal-recessive skeletal dysplasias (*ACAN*, *NPR2*), confirming a dosage effect of cartilage matrix proteins in growth development.[26,27,29,30] In our exome study, *ACAN* was the most commonly mutated known short stature–associated gene with a frequency of 2.5% (**Supplementary Table S2**), whereas the previously reported most common single-gene defect affects *SHOX* with a frequency of 2.4%.[8]

An important aspect of the clinical application of whole-exome sequencing in short stature concerns prognosis, prevention, and treatment (**Table 4** and **Supplementary Table S6**). In 11.5% of the families, the identification of the molecular cause of the disease by exome sequencing prompted further preventive action. The girl diagnosed with a mutation in *PDE3A* might benefit from current targeted treatment with phosphodiesterase inhibitors to reduce her high risk of life-threatening coronary artery disease and essential hypertension after puberty.[35] Also 1.5% of the affected individuals became eligible for targeted treatment for growth retardation itself. Finally, 5% of the patients could benefit from treatment or screening for associated malformations. None of these clinical applications were considered prior to genetic diagnosis by exome sequencing, but they have now been applied to the patients' care.

ORIGINAL RESEARCH ARTICLE

In conclusion, we demonstrated that systematic phenotyping combined with targeted genetic testing and exome sequencing increases the diagnostic yield in short stature up to 33% with concomitant improvement in treatment and prevention. As height has a proposed heritability of about 80%, and as the ongoing rate of discovery in other entities suggests,[40] we expect that future identification of potential candidate genes, as well as their analysis in additional patients, will increase the diagnostic yield.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

DISCLOSURE

The authors declare no conflict of interest.

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