Genetic findings in short Turkish children born to consanguineous parents

Hormone Research in Paediatrics

Manuscript:	HRP-2024-2-18/R2 RESUBMISSION
Title:	Genetic findings in short Turkish children born to consanguineous parents
Authors(s):	Sjoerd D. Joustra (Corresponding Author), Emregul Isik (Co-author), Jan M. Wit (Co-author), Gonul Catli (Co-author), Ahmet Anik (Co-author), belma haliloglu (Co-author), Nurgun Kandemir (Co-author), Elif Ozsu (Co-author), Yvonne M.C. Hendriks (Co-author), Christiaan de Bruin (Co-author), Sarina G. Kant (Co-author), Angel Campos-Barros (Co-author), Rachel C. Challis (Co-author), David Parry (Co-author), Margaret E. Harley (Co-author), Andrew Jackson (Co-author), Monique Losekoot (Co-author), Hermine A van Duyvenvoorde (Co-author)
Keywords:	Consanguinity, copy number variants, growth hormone, Short stature, single nucleotide variants
Type:	Research Article

Prof.dr. Stefano Cianfarani

Editor-in-chief, Hormone Research in Paediatrics

Manuscript ID: HRP-2024-2-18

May 23rd, 2024

Re: Rebuttal letter for manuscript *Genetic findings in short Turkish children born to consanguineous parents*

We thank the reviewers for their reconsideration of our manuscript and the additional constructive comments to the revised version. Our point for point responses to the comments are shown below. The revised manuscript is submitted separately.

Reviewer #1

General remark

The review of MS has addressed all questions adequately and only a few minor modifications are necessary.

Comment 1

Table 1, patient 4. Even though the use of PM1 (Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation) for this variant is doubtful, PM1, PM2 and PP3 (supp) results in a classification as VUS and not as LP.

Response 1

Based on the comment by the reviewer, we carefully re-evaluated the variant in patient 4, and discussed it in our multi-disciplinary team. The phenotype is archetypical for Laron syndrome, with severe short stature, profoundly low IGF-I levels, no response in an IGF-I generation test, and a normal stimulated GH peak. We therefore added PP4 moderate to the classification (PM2, PM3_sup, PP3, PP4_mod). Based on this classification, the variant may be considered as being between VUS and likely pathogenic. In combination with finding the same variant in his brother, who also has the typical phenotype of Laron syndrome, our team is convinced the variant should be classified as likely pathogenic. We hope the reviewer and editor follow our reasoning and allow our view of this variant to be presented in the manuscript.

Comment 2

Table 1, patient 12. The ACMG classification should be reviewed, as PVS1 was used for the two missense variants (PVS1: null variant (nonsense, frameshift, canonical +- 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease). In this case, it may be appropriate to use criterion PP4: "Patient's phenotype or family history is highly specific for a disease with a single genetic etiology". Even so, I believe that the variant will be classified as VUS.

Response 2

The reviewer has an excellent point and we are thankful for this observation. It allowed us to notice that there has been an unfortunate copy-paste error in Table 1 for this variant. Indeed, PVS1, PM2, PM3_sup would mean the variant is classified as a VUS. However, the original and correct classification for this variant is PM1, PM2, PM3, PP3. This leads to a likely pathogenic classification, for both *IGFALS* variants. We corrected Table 1.

Comment 3

Table 1 [we think Table 2 was meant], *patient 19. The format of the pathogenicity classification must be uniform and in accordance with the rest of the variants.*

Response 3

The variant in patient 19 is a deletion of one exon. Therefore, the ACMG criteria for CNVs instead of SNVs were used (1). This explains the difference in reporting format.

1. Rooney Riggs et al., Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen), Genet Med. 2020 Feb;22(2):245-257.

Genetic findings in short Turkish children born to consanguineous parents

Sjoerd D. Joustra[1]*, Emregul Isik[2]*, Jan M. Wit[1]*, Gonul Catli[3,4], Ahmet Anik[5], Belma Haliloglu[6], Nurgun Kandemir[7], Elif Ozsu[8], Yvonne M.C. Hendriks[9], Christiaan de Bruin[1], Sarina G. Kant[10], Angel Campos-Barros[11], Rachel C. Challis[12], David Parry[12], Margaret E. Harley[12], Andrew Jackson[12], Monique Losekoot[9#], Hermine A. van Duyvenvoorde[9#]

[1]Willem-Alexander Children's Hospital, Department of Paediatrics, Division of Pediatric Endocrinology, Leiden University Medical Center, the Netherlands

[2]Department of Paediatrics, Ankara Bilkent City Hospital, Ankara, Turkey

[3]Department of Paediatric Endocrinology, Izmir Katip Celebi University Faculty of Medicine, Izmir, Turkey

[4]Department of Paediatric Endocrinology, Istinye University Faculty of Medicine, Istanbul, Turkey

[5]Department of Paediatric Endocrinology, Dokuz Eylul University, Izmir, Turkey

[6]Department of Paediatric Endocrinology and Diabetology, Marmara University School of Medicine, Istanbul, Turkey

[7]Department of Paediatric Endocrinology, Hacettepe University, Faculty of Medicine, Ankara, Turkey

[8]Department of Paediatric Endocrinology and Diabetes, University of Ankara, Ankara, Turkey

[9]Department of Clinical Genetics, Leiden University Medical Centre, Leiden, Netherlands

[10]Department of Clinical Genetics, Erasmus Medical Centre, Rotterdam, Netherlands

[11] Institute of Medical & Molecular Genetics (INGEMM), IdiPAZ, Hospital Universitario La Paz, and Rare Diseases Biomedical Research Network (CIBERER; U 753), ISCIII, Madrid, Spain

[12]MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom

*These authors contributed equally to this paper

#These authors share last authorship

Short title: Genetic causes of short stature in consanguinity

Corresponding author and reprint requests:

Dr. S.D. Joustra, sdjoustra@lumc.nl

Keywords: short stature, consanguinity, single nucleotide variants, copy number variants, growth hormone

Abstract

Introduction

The diagnostic yield of genetic analysis in the evaluation of children with short stature depends on associated clinical characteristics, but the additional effect of parental consanguinity has not been well documented.

Methods

This observational case series of 42 short children from 34 consanguineous families was collected by six referral centres of paediatric endocrinology (inclusion criteria: short stature and parental consanguinity). In eighteen patients (12 families, Group 1), the clinical features suggested a specific genetic defect in the growth hormone (GH)-insulin-like growth factor I (IGF-I) axis, and a candidate gene approach was used. In others (Group 2) a hypothesis-free approach was chosen (gene panels, microarray analysis, and whole-exome sequencing), further subdivided into 11 patients with severe short stature (height <-3.5 SDS) and microcephaly (head circumference <-3.0 SDS) (group 2a), 10 patients with syndromic short stature (group 2b) and were 3 patients with nonspecific isolated GH deficiency (group 2c).

Results

In all 12 families from group 1, (likely) pathogenic variants were identified in *GHR*, *IGFALS*, *GH1*, and *STAT5B*. In 9/12 families from group 2a, variants were detected in *PCNT*, *SMARCAL1*, *SRCAP*, *WDR4* and *GHSR*. In 5/9 families from group 2b, variants were found in *TTC37*, *SCUBE3*, *NSD2*, *RABGAP1*, and 17p13.3 microdeletions. In group 2c no genetic cause was found. Homozygous, compound heterozygous and heterozygous variants were found in 21, 1 and 4 patients, respectively.

Conclusion

Genetic testing in short children from consanguineous parents has a high diagnostic yield, especially in cases of severe GH deficiency or insensitivity, microcephaly, and syndromic short stature.

Introduction

The diagnostic approach of a child presenting with short stature, defined as a height standard deviation score (SDS) below -2 and/or decreased growth velocity, has changed considerably over the last two decades. Traditionally, the focus was on detecting dysmorphic syndromes or disorders of the endocrine or other organ systems. In most cases no definite diagnosis could be made, so that the attribution of descriptive diagnostic labels like “children born small for gestational age with failure of catch-up growth” or “idiopathic short stature”, which includes familial idiopathic short stature and constitutional delay of growth and puberty [1], would often mark the end of the diagnostic work-up [2]. In the last two decades, many new genetic tools have become available, which has led to the discovery of numerous novel genetic defects associated with short stature [3-13]. It has also become clear that the prevalence of monogenic growth disorders is much higher than previously assumed, and that their phenotypic variability is substantial [8, 14, 13].

In some cases, the clinical and biochemical features of a short patient are so specific or indicative for a certain condition, that the clinician can use the traditional candidate-gene approach. However, a gene panel or an exome-/genome-wide approach is often more successful and cost-effective [9, 11]. Until recently, this hypothesis-free approach consisted of consecutive analysis of copy number variants (CNVs) through microarrays and pathogenic single nucleotide variants (SNV) through massive parallel sequencing using a gene panel or whole exome sequencing (WES). Present technology has made it possible to perform both SNV and CNV analysis on massive parallel sequencing data [15, 16].

The current challenge is to decide which children with short stature should be tested for genetic causes. Several clues from the clinical assessment can increase the pre-test likelihood of a monogenic defect or causal CNV [3, 17]. The diagnostic yield is relatively high if the short child presents with additional clinical features, such as (facial) dysmorphisms, body disproportion, congenital anomalies, neurodevelopmental disorders, microcephaly or relative macrocephaly, signs of skeletal dysplasia, and severe short stature [3, 8, 10, 18, 17, 12, 13]. Abnormal biochemical findings can also suggest a monogenic defect, such as in patients with severe growth hormone (GH) deficiency or insensitivity [19, 20, 13]. Furthermore, an adequate assessment of the family history is important. A dominant pattern of inheritance is suggestive for haploinsufficiency of a gene involved in growth plate biology, such as *IGF1R, SHOX, NPR2, ACAN, NPPC, IHH* and genes associated with collagenopathies [3, 21, 6, 22, 17, 23, 14]. In contrast, in the offspring of consanguineous parents the likelihood of a recessive condition is expected to be increased.

The purpose of the current paper is to describe the yield of extensive genetic testing in diagnosing the cause of short stature in 42 children from 34 consanguineous families.

Material and Methods

Subjects

Out of a total of 57 patients from Turkish referral centres of paediatric endocrinology, we included 42 patients (from 34 families) into this study who complied with the inclusion criteria: age below 18 years, short stature, parental consanguinity, and at least one additional feature associated with increased risk for a genetic defect (Figure 1), e.g. GH deficiency, height <-3.5 SDS, microcephaly, or syndromic features. These patients were identified in six Turkish paediatric endocrinology centres and discussed with the Leiden Genetics of Growth Expertise Center of the Leiden University Medical Center (LUMC) in the Netherlands before DNA was submitted.

Data on weight, length and head circumference at birth, height, body mass index (BMI) and sitting height/height were expressed as SDS for age and sex based on Turkish reference data [24, 25]. Head circumference was expressed as SDS for Dutch children [26]. Serum IGF-I and IGFBP-3 were expressed as SDS for local standards. The GH peak at GH stimulation (GHmax) was expressed in ng/mL. In 9 patients an IGF-I generation test was performed, according to local protocols.

Patients were divided into two groups. Group 1 consisted of patients in whom the clinical features suggested a specific genetic defect in the GH-IGF-I axis, so that a candidate gene approach was chosen. These DNA samples were investigated in the Laboratory for Diagnostic Genome analysis in the LUMC (Leiden, the Netherlands). Group 2 consisted of short patients with at least one additional clinical feature and tested with a hypothesis-free approach. This group was further subdivided into three subgroups. Subgroup 2a included patients with severe short stature (height <-3.5 SDS) and microcephaly (head circumference <-3.0 SDS). Most of these were investigated in the MRC Human Genetics Unit of the University of Edinburgh in the United Kingdom with a gene panel (see Supplementary data). Subgroup 2b included non-GH deficient subjects with syndromic short stature, without clues for a specific clinical diagnosis. They were analysed in the LUMC in a step-wise approach using SNP-array, custom gene panels using human phenotype ontology terms, and exome-wide analysis of WES data of the patient and both parents (trio WES). Subgroup 2c consisted of three patients with isolated GH deficiency in whom no specific gene defect was apparent at clinical evaluation ('nonspecific GH deficiency'). Their DNA was sent to the Molecular Endocrinology Division of the Institute of Medical and Molecular Genetics at the La Paz University Hospital in Madrid (Spain) for trio WES and CNV analysis.

Genetic diagnostic procedures

Genomic analyses were performed using DNA samples obtained from leukocytes. The candidate gene approach was performed using Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA). Target enrichment kits, sequencing platforms, and WES statistics are reported in the Supplemental data. Data processing, variant filtering and classification were performed using laboratory specific standard procedures (see Supplemental data). Co-segregation analysis was performed by Sanger sequencing in some families (primer sequences available on request).

Results

In group 1, the candidate gene approach led to a genetic diagnosis in all 12 families (18 patients) (Table 1). In group 2, a genetic diagnosis was established in 14 out of 22 families (64%) (16 out of 24 patients (67%). In subgroup 2a, the genetic cause was established in nine out of ten families, and in subgroup 2b in five out of nine families (Table 2). In the three patients with nonspecific isolated GH deficiency (subgroup 2c), no pathogenic variants that could explain the phenotype were identified. The majority of genetic defects were homozygous (21/26), one was compound heterozygous and four were heterozygous.

Group 1; subjects suspected of a specific genetic defect in the GH-IGF axis

Clinical and genetic results are presented in Supplementary Table S1 and Table 1, respectively.

In two siblings in family 1 with the classical biochemical presentation of GH deficiency type 1A and neonatal hypoglycaemia [27], a previously described *GH1* deletion of exons 3-5 was found by MLPA [28]. Remarkably, in the younger sibling microcephaly was noted. Treatment with recombinant human GH (rhGH) was initiated, but the patients were lost to follow-up.

In nine patients suspected for Laron syndrome from five unrelated families (families 2-6), four (likely) pathogenic *GHR* variants were found. Families 2 and 3 live in the same city and carry the same p.Asn115Thr variant that was previously identified in Saudi-Arabia [29]. The families were unaware of common inheritance. The novel p.Val303Asp variant in family 4 is located in the highly conserved C-terminal domain of the protein and was assigned likely pathogenic. The p.Ser58Leu variant in family 5 has been described previously [30]. All patients had very low serum levels of IGF-I and IGFBP-3, but baseline serum GH was elevated in only 3 patients. In families 2, 3, 4 and 6, mean height SDS was -3.4 (range -4.3 to -2.1, n=7), mean head circumference (HC) -1.55 (range -2.5 to -1.1, n=6) and HC SDS minus height SDS 1.6 (range -0.1 to 3.3, n=6). The two siblings in family 5 had far more severe short stature (height -7.2 and -11.2 SDS, HC unavailable).

Patient 6 showed a pseudoexon inclusion in *GHR* caused by a homozygous deep-intronic variant (c.618+792A>G), consistent with a reportedly relatively mild form of Laron syndrome [31]. His

sensorineural deafness was caused by a homozygous pathogenic nonsense variant in *ADGRV1* (NM_032119.4, c.7446C>G, p.Tyr2482*) confirming the diagnosis of Usher syndrome type 2C (MIM #605472). His hypothyroidism may be associated with the heterozygous variant of unknown significance (VUS) in *DUOX1* (NM_175940.3, c.2036G>A, p.Arg679His.

Patient 7 presented at the age of 17 years with short stature, ichthyosis, midface hypoplasia, frontal bossing and hyperprolactinemia, suggestive for a bi-allelic *STAT5B* variant, despite the absence of any history of frequent infections or lung problems. She showed a homozygous novel truncating variant in *STAT5B*. Further clinical details of this patient were recently reported [32]. She also presented with normogonadotrophic primary amenorrhoea at Tanner 4, which has not been observed in previously reported patients. We postulate that this may an additional clinical feature of this condition, possibly related to her hyperprolactinemia (225 ng/mL, reference range 2.5-25 ng/mL).

Offspring of families 8 to 12 were diagnosed with acid-labile subunit deficiency caused by four likely pathogenic homozygous or compound heterozygous *IGFALS* variants. Clinical details of these patients and their relatives were reported previously [33].

Subgroup 2a; subjects with severe short stature (height <-3.5 SDS) and microcephaly (head circumference <-3.0 SDS)

Eleven patients from 10 families presented with severe short stature and microcephaly. A summary of the clinical data is presented in Supplementary Table S2 and in the Supplementary clinical information. Genetic results are shown in Table 2.

Patients 13 and 14 from two reportedly unrelated families living in the same city were homozygous for an identical pathogenic variant in *SMARCAL1*, consistent with the diagnosis of Schimke immuno-osseous dysplasia (MIM #242900). The variant was previously reported [34-38].

In patient 15 a previously reported heterozygous pathogenic *SRCAP* variant was detected [39-44], consistent with Floating-Harbor syndrome (MIM #136140).

In 5 patients from families 16-19, homozygosity was shown for four different (likely) pathogenic *PCNT* variants, with the typical clinical features of microcephalic osteodysplastic primordial dwarfism type 2 (MOPD2, MIM #210720). The variants in patients 16, 18 and 19 are novel, while the variant in family 17 has been reported previously in two Turkish patients [45] and elsewhere [46, 47].

In patient 20 a novel homozygous *WDR4* variant was detected. This gene encodes a tRNA methyltransferase, and homozygous loss of function is associated with primordial dwarfism ("microcephaly, growth deficiency, seizures, and brain malformations", MIGSB, MIM #618346) [48-52, 13].

In patient 21 we detected a heterozygous VUS in *GHSR*, which was also present in his short mother and sister, and not in the normal statured father, brother and sister. The phenotype and chemotype of patients carrying *GHSR* variants is diverse, including normal or delayed pubertal development and normal or low GHmax in GH stimulation tests [20]. Although limited information is available about Tanner stages of our patient, delayed puberty is very likely based on the shape of the growth curve, considerable bone age delay (2.7 years at discontinuation of 9 months of rhGH treatment) and an increase of height SDS in late adolescence resulting in an adult height of -2.0 SDS.

In patient 22, no genetic abnormality was detected in any gene associated with primordial dwarfism, nor in chromosomal microarray analysis.

Subgroup 2b; patients with syndromic short stature

Ten patients from 9 families presented with syndromic short stature. A summary of the clinical data are presented in Supplementary Table S3 and in the Supplementary clinical information. Genetic results are shown in Table 2.

In patient 23, born extremely small for gestational age, SNP-array analysis showed two novel 17p13.3 microdeletions separated by a small non-deleted region of 101.5 kb. These heterozygous deletions were not found in the mother's DNA (father's DNA was unavailable). The terminal deletion (minimal size 1.9 Mb, 1984 probes, from 525 bp to 1.922.715 bp) contains 31 protein coding genes including *YWHAE* (also known as 14-3-3 epsilon) and *CRK*. The interstitial deletion (minimal size 238.5 kb, 420 probes, from 2.024.217 bp tot 2.262.703 bp) contains 4 protein coding genes (*SMG6*, *SGSM2*, *SRR* and *TSR1*). Both deletions have not been described as genomic variants in the population. The deletions do not contain the LIS1 gene (*PAFAH1B1*). The short arm of chromosome 17 is characterized by a high density of low copy repeats, creating the opportunity for non-allelic homologous recombination to occur. There are three classes of contiguous gene deletion syndromes known in this region: 1) isolated lissencephaly sequence: *PAFAH1B1* deleted, *YWHAE* not deleted (LIS1; MIM #607432); 2) *YWHAE* and/or *CRK* deleted, *PAFAH1B1* not deleted; and 3) Miller-Dieker syndrome: both areas deleted (MIM #247200). Our patient is an example of the second class, so far observed in 19 patients [53]. Her clinical features are similar to those described for other patients with a similar genotype (for details, see Supplementary Information). A recent paper showed that *YWHAE* loss-of-function variants cause a neurodevelopmental disease with brain abnormalities, and that individuals with variants affecting *YWHAE* alone have milder features than those with larger deletions [54]. Since linear growth is normal in individuals with isolated *YWHAE* defects [54] and severely decreased if *CRK* is deleted [55, 56], *CRK* deletion seems responsible for the short stature observed in class 2 and 3 deletions.

In patient 24, genetic analysis showed a homozygous pathogenic *TTC37* variant, reported previously [57, 58], consistent with Trichohepatoenteric syndrome 1 (THES1, MIM #222470).

In the two siblings in family 25, we discovered a homozygous novel *SCUBE3* variant, which at that time had not been described as a cause of short stature. In an international research project, eight more families were identified, and functional studies were performed in *Scube3-/-* mice. This led to the identification of a novel genetic cause of dysmorphic short stature, now called "Short Stature, Facial Dysmorphism, And Skeletal Anomalies With Or Without Cardiac Anomalies 2" (SSFSC2, MIM #619184) [59].

In patient 26 a *de novo* frameshift variant in *NSD2* was detected. *NSD2* loss-of-function variants cause decreased methylation activity and are associated with a distinct developmental phenotype partially overlapping with Wolf-Hirschhorn syndrome (WHS) (4p16.3 deletion syndrome) [60]. In a recent paper on a comprehensive series of 18 patients carrying heterozygous missense, elongating, or truncating *NSD2* variants, the core NSD2-associated phenotype was shown to include mostly mild developmental delay, prenatal-onset growth retardation, low BMI, and characteristic facial features distinct from WHS. The authors proposed that NSD2 deficiency may be named Rauch–Steindl syndrome after the delineators of this phenotype [60]. Most patients display mild cognitive impairment, but some go to a regular school as did our patient. Formal IQ testing was not performed.

In patient 27, we found a novel homozygous variant in *RABGAP1*, which was recently described as the cause of a neurodevelopmental syndrome in five patients (carrying three different variants) with intellectual disability, microcephaly, bilateral sensorineural hearing loss, seizures and overlapping dysmorphic features [61].

In patients 28-31, no definitive genetic diagnosis could be made. For clinical and genetic findings, see Supplementary Information.

Subgroup 2c; subjects with nonspecific isolated GH deficiency

Characteristics of the three patients with isolated GH deficiency are shown in Supplementary Table S4 and the Supplementary clinical information. The results of the WES analysis was non-conclusive. Patient 34 presented with GH deficiency plus widespread severe eczema, onychomycosis, cheilitis, peeling skin and acral punctate keratosis. His skin phenotype is likely caused by a homozygous nonsense mutation in *CAST* as revealed by duo WES analysis of the proband and his mother [62]. There is no known association between variants in *CAST* and GH deficiency or short stature.

Discussion

We performed extensive genetic analysis in 42 short children from 34 Turkish consanguineous families and observed a high diagnostic yield: in 26 out of 34 families (76%) a genetic cause was found (34 out of 42 short children, 81%). The candidate gene approach resulted in a genetic diagnosis in all 12 families (group 1), with pathogenic variants in several genes in the GH-IGF axis: *GH1* (1 family), *GHR* (5 families), *STAT5B* (1 family) and *IGFALS* (5 families). A gene panel in patients with microcephalic primordial dwarfism (subgroup 2a) resulted in a positive diagnosis in 9 out of 10 families. In patients with syndromic short stature (subgroup 2b) a genetic cause was found in 5 out of 9 families. In none of the three patients with nonspecific isolated GH deficiency (subgroup 2c) a genetic cause was found.

Regarding genetic defects of the GH-IGF axis (group 1), we show that based on clinical features, growth pattern and laboratory investigations (serum IGF-I, IGFBP-3, prolactin, GH stimulation testing and IGF generation test), the most likely candidate gene can be identified. Severe postnatal short stature in a child with normal birth weight, neonatal hypoglycaemia, unmeasurable serum GH before and after stimulation, and very low serum IGF-I and IGFBP-3, strongly point into the direction of a *GH1* deletion (Family 1). Most of our patients with Laron syndrome showed the classical phenotype and chemotype, but notably baseline serum GH was not elevated in most patients. In the patient with a novel homozygous *STAT5B* variant the eczema and elevated serum prolactin in combination with the very low serum IGF-I and IGFBP-3 pointed into the direction of this disorder, but the low baseline and stimulated GH secretion and mild clinical presentation were unexpected. Patients with bi-allelic *IGFALS* variants are characterized by a modest short stature and a serum IGFBP-3 SDS that is considerably lower than IGF-I SDS [33].

The patients with confirmed Laron syndrome had an extremely variable height SDS (range -2.1 to -11.2 SDS), consistent with previous observations [63]. Two out of six patients were borderline microcephalic (-2.0 and -2.5 SDS), and patient 3b had a similar SDS for height and head circumference, in contrast to the classical presentation of Laron syndrome. A recent study exemplifies that exome sequencing in these patients might reveal additional genetic defects responsible for microcephaly [64], as consanguinity increases the likelihood of multiple recessive genetic conditions. While serum IGF-I and IGFBP-3 concentrations were extremely low in all patients, baseline serum GH concentrations were elevated in only three of them.

As expected for offspring of consanguineous couples, most patients were homozygous carriers of gene variants (21 families). However, one should note that in 4 families the patient's short stature was caused by a heterozygous defect and in one family by compound heterozygosity. This finding illustrates that in offspring of consanguineous marriages pathogenic genetic aberrations may not necessarily present as homozygous variants, in line with a previous report in a Saudi cohort [65].

Our study also shows that several patients not only carry gene variants responsible for short stature, but also have (homozygous) variants that explain additional clinical features. An example is patient 6 who carries homozygous variants in *GHR* as well as in *ADGRV1* (responsible for his sensorineural deafness).

Regarding our patients in whom targeted gene panels or trio WES analysis did not yield a genetic diagnosis, we speculate that these may carry variants in genes which have not been associated with short stature yet, or genetic defects outside of the coding areas of the genome. In the future, whole genome sequencing, RNA sequencing (including analysis of microRNAs and long-noncoding RNAs) or epigenetic analyses may be needed to establish the diagnosis.

Regarding the clinical benefit of genetic testing in severe growth disorders, there are at least four reasons why identification of rare monogenic causes is beneficial [3]. First, identification of a molecular aetiology can end the diagnostic workup for the patient and provide the family with an answer as to why their child is not growing normally. Second, the genetic diagnosis may alert the clinician to medical comorbidities for which the patient is at risk. This does not only benefit the patient, but may also alert the affected relatives to such comorbidities, for example early-onset osteoarthritis and degenerative disc disease if an *ACAN* variant is identified [66]. Third, determination of a molecular aetiology is invaluable for genetic counselling. Fourth, the genetic aetiology may have implications for therapy, in particular whether rhGH treatment may be efficacious and safe. For the patients with disorders of the GH-IGF axis, there are clear therapeutic consequences: rhGH treatment for those with a *GH1* or *GHSR* defect, recombinant human IGF-I (rhIGF-I) treatment for those homozygous for *GHR* or *STAT5B* variants, and a contraindication of rhGH or rhIGF-I in those with bi-allelic *IGFALS* variants because of the expected poor growth response [67].

We suggest two more reasons why identification of rare monogenic causes can be beneficial. First, increasing the number of patients with short stature who undergo genetic testing will lead to a better insight into the broadness of the spectrum of clinical phenotypes associated with genetic syndromes, which will in turn help the identification of future cases. An example from our patients is the elaborate description of the phenotype of patients carrying bi-allelic *IGFALS* variants [33]. Another example is the observation that short stature is a clinical feature associated with the recently described syndrome associated with bi-allelic *RABGAP1* variants [61]. Second, genetic testing can lead to the uncovering of novel syndromes, such as the syndrome caused by bi-allelic *SCUBE3* variants which we discovered in the two affected siblings in family 24, and reported jointly with other investigators [59].

There is also a potential benefit of describing the clinical features of patients in whom genetic findings are still uncertain, as for example the *LARP7* variant found in patient 30 and the *SPATA5* variant we detected in patient 31 (Supplemental Information). If in the future other patients with a similar phenotype are found who carry a variant in one of these genes, this may lead to the identification of a novel genetic cause of short stature. Lastly, we would like to emphasize that the observed frequency of genetic causes of short stature is dependent on the population that is studied, and likely differs between patients from different ancestries.

In conclusion, thorough genetic analysis in short children from consanguineous parents has a high diagnostic yield, especially in case of severe GH deficiency and insensitivity, microcephaly, and syndromic short stature. Diagnosing these patients has important clinical consequences and provides more insights in the scope of the clinical features associated with monogenic causes of short stature. While most patients carried homozygous genetic defects, heterozygous and compound heterozygous defects were also found.

Statements

Acknowledgement

We are grateful to the patients and their caregivers for participating in this study.

Statement of Ethics

Ethical approval was obtained in the Ankara Bilkent City Hospital in Ankara, Turkey (document number E2.22.2146). All patients that were 12 years and older, and all parents of patients younger than 16 years old, gave written informed consent for the collection of data, and publication of their medical case.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

Work in the Jackson lab was supported by the European Union's Horizon 2020 research and innovation program ERC Advanced Grant (grant agreement 788093) and by a UK Medical Research Council (MRC) Human Genetics Unit core grant (MC_UU_00007/5). A.C-B was supported by grant PI18/00402, Instituto de Salud Carlos III (ISCIII, Madrid, Spain/ European Regional Development Fund, ERDF).

Author Contributions

S.J.D. was involved in the genetic analysis of several patients and led the writing process. E.I. collected most patients and commented upon the subsequent versions of the manuscript. J.M.W. analysed the patients' clinical features, organised genetic testing and prepared consecutive versions of the manuscript. G.C., A.A, B.H., N.K. and E.O. collected clinical information from the patients they referred. Y.M.C.H. interpreted the genetic findings. C.d.B. and S.G.K. were involved in discussions on the clinical and genetic findings of the patients. A.C-B was responsible for the genetic analysis of subgroup 2c. R.C.C, D.P., M.E.H and A.J. were responsible for the genetic analysis of most patients in subgroup 2b. M.L and H.A.v.D. were responsible for the genetic analysis of all patients tested in Leiden and supervised consecutive versions of the manuscript. All authors consented to submission of the manuscript.

Data Availability Statement

WES datasets have not been deposited in a public repository due to privacy and ethical restrictions but are available from the corresponding author on request.

References

1. Quigley CA, Ranke MB. International Classification of Pediatric Endocrine Diagnoses. Rotterdam: Growth Analyser; 2016.
2. Rapaport R, Wit JM, Savage MO. Growth failure: 'idiopathic' only after a detailed diagnostic evaluation. Endocr Connect. 2021 Mar;10(3):R125-R38.
3. Dauber A, Rosenfeld RG, Hirschhorn JN. Genetic evaluation of short stature. J Clin Endocrinol Metab. 2014 9/2014;99(9):3080-92.
4. Baron J, Savendahl L, De Luca F, Dauber A, Phillip M, Wit JM, et al. Short and tall stature: a new paradigm emerges. Nat Rev Endocrinol. 2015 Dec;11(12):735-46.
5. Wit JM, Oostdijk W, Losekoot M, van Duyvenvoorde HA, Ruivenkamp CA, Kant SG. MECHANISMS IN ENDOCRINOLOGY: Novel genetic causes of short stature. Eur J Endocrinol. 2016 Apr;174(4):R145-73.
6. Jee YH, Andrade AC, Baron J, Nilsson O. Genetics of Short Stature. Endocrinol Metab Clin North Am. 2017 Jun;46(2):259-81.
7. Finken MJJ, van der Steen M, Smeets CCJ, Walenkamp MJE, de Bruin C, Hokken-Koelega ACS, et al. Children Born Small for Gestational Age: Differential Diagnosis, Molecular Genetic Evaluation, and Implications. Endocr Rev. 2018 Dec 1;39(6):851-94.

8. Hauer NN, Popp B, Schoeller E, Schuhmann S, Heath KE, Hisado-Oliva A, et al. Clinical relevance of systematic phenotyping and exome sequencing in patients with short stature. Genet Med. 2018 Jun;20(6):630-38.
9. Dauber A. Genetic Testing for the Child With Short Stature-Has the Time Come To Change Our Diagnostic Paradigm? J Clin Endocrinol Metab. 2019 Jul 1;104(7):2766-69.
10. Freire BL, Homma TK, Funari MFA, Lerario AM, Vasques GA, Malaquias AC, et al. Multigene Sequencing Analysis of Children Born Small for Gestational Age With Isolated Short Stature. J Clin Endocrinol Metab. 2019 Jun 1;104(6):2023-30.
11. Andrade NLM, Funari MFA, Malaquias AC, Collett-Solberg PF, Gomes N, Scalco R, et al. Diagnostic yield of a multigene sequencing approach in children classified as idiopathic short stature. Endocr Connect. 2022 Dec 1;11(12).
12. Freire BL, Homma TK, Lerario AM, Seo GH, Han H, de Assis Funari MF, et al. High frequency of genetic/epigenetic disorders in short stature children born with very low birth weight. Am J Med Genet A. 2022 Sep;188(9):2599-604.
13. Li X, Yao R, Chang G, Li Q, Song C, Li N, et al. Clinical Profiles and Genetic Spectra of 814 Chinese Children With Short Stature. J Clin Endocrinol Metab. 2022 Mar 24;107(4):972-85.
14. Plachy L, Dusatkova P, Maratova K, Petruzelkova L, Elblova L, Kolouskova S, et al. Familial Short Stature-A Novel Phenotype of Growth Plate Collagenopathies. J Clin Endocrinol Metab. 2021 May 13;106(6):1742-49.
15. Hehir-Kwa JY, Tops BBJ, Kemmeren P. The clinical implementation of copy number detection in the age of next-generation sequencing. Expert Rev Mol Diagn. 2018 Oct;18(10):907-15.
16. Royer-Bertrand B, Cisarova K, Niel-Butschi F, Mittaz-Crettol L, Fodstad H, Superti-Furga A. CNV Detection from Exome Sequencing Data in Routine Diagnostics of Rare Genetic Disorders: Opportunities and Limitations. Genes (Basel). 2021 Sep 16;12(9).
17. Wit JM, Kamp GA, Oostdijk W, on behalf of the Dutch Working Group on T, Diagnosis of Growth Disorders in C. Towards a Rational and Efficient Diagnostic Approach in Children Referred for Growth Failure to the General Paediatrician. Horm Res Paediatr. 2019;91(4):223-40.
18. Homma TK, Freire BL, Honjo Kawahira RS, Dauber A, Funari MFA, Lerario AM, et al. Genetic Disorders in Prenatal Onset Syndromic Short Stature Identified by Exome Sequencing. J Pediatr. 2019 Dec;215:192-98.
19. Alatzoglou KS, Dattani MT. Genetic forms of hypopituitarism and their manifestation in the neonatal period. Early Hum Dev. 2009 11/2009;85(11):705-12.

20. 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.
21. Wit JM, de Luca F. Atypical defects resulting in growth hormone insensitivity. Growth Horm IGF Res. 2016 Jun;28:57-61.
22. Plachy L, Strakova V, Elblova L, Obermannova B, Kolouskova S, Snajderova M, et al. High Prevalence of Growth Plate Gene Variants in Children With Familial Short Stature Treated With GH. J Clin Endocrinol Metab. 2019 Oct 1;104(10):4273-81.
23. Plachy L, Dusatkova P, Maratova K, Petruzelkova L, Zemkova D, Elblova L, et al. NPR2 Variants Are Frequent among Children with Familiar Short Stature and Respond Well to Growth Hormone Therapy. J Clin Endocrinol Metab. 2020 Mar 1;105(3).
24. Bundak R, Bas F, Furman A, Gunoz H, Darendeliler F, Saka N, et al. Sitting height and sitting height/height ratio references for Turkish children. Eur J Pediatr. 2014 Jul;173(7):861-9.
25. Neyzi O, Bundak R, Gokcay G, Gunoz H, Furman A, Darendeliler F, et al. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol. 2015 Dec;7(4):280-93.
26. Fredriks AM, Van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, et al. Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res. 2000 3/2000;47(3):316-23.
27. Alatzoglou KS, Turton JP, Kelberman D, Clayton PE, Mehta A, Buchanan C, et al. Expanding the spectrum of mutations in GH1 and GHRHR: genetic screening in a large cohort of patients with congenital isolated growth hormone deficiency. J Clin Endocrinol Metab. 2009 9/2009;94(9):3191-99.
28. Kale S, Gada JV, Jadhav S, Lila AR, Sarathi V, Budyal S, et al. Genetic spectrum and predictors of mutations in four known genes in Asian Indian patients with growth hormone deficiency and orthotopic posterior pituitary: an emphasis on regional genetic diversity. Pituitary. 2020 Dec;23(6):701-15.
29. Al-Ashwal AA, Al-Sagheir A, Ramzan K, Al-Owain M, Allam R, Qari A, et al. Clinical, Endocrine, and Molecular Genetic Analysis of a Large Cohort of Saudi Arabian Patients with Laron Syndrome. Horm Res Paediatr. 2017;88(2):119-26.
30. Sobrier ML, Dastot F, Duquesnoy P, Kandemir N, Yordam N, Goossens M, et al. Nine novel growth hormone receptor gene mutations in patients with Laron syndrome. J Clin Endocrinol Metab. 1997 Feb;82(2):435-7.

31. Metherell LA, Akker SA, Munroe PB, Rose SJ, Caulfield M, Savage MO, et al. Pseudoexon activation as a novel mechanism for disease resulting in atypical growth-hormone insensitivity. Am J Hum Genet. 2001 9/2001;69(3):641-46.
32. Catli G, Gao W, Foley C, Ozyilmaz B, Edeer N, Diniz G, et al. Atypical STAT5B deficiency, severe short stature and mild immunodeficiency associated with a novel homozygous STAT5B Variant. Mol Cell Endocrinol. 2022 Oct 17;559:111799.
33. Isik E, Haliloglu B, van Doorn J, Demirbilek H, Scheltinga SA, Losekoot M, et al. Clinical and biochemical characteristics and bone mineral density of homozygous, compound heterozygous and heterozygous carriers of three novel IGFALS mutations. Eur J Endocrinol. 2017 Jun;176(6):657-67.
34. Boerkoel CF, Takashima H, John J, Yan J, Stankiewicz P, Rosenbarker L, et al. Mutant chromatin remodeling protein SMARCAL1 causes Schimke immuno-osseous dysplasia. Nat Genet. 2002 Feb;30(2):215-20.
35. Elizondo LI, Cho KS, Zhang W, Yan J, Huang C, Huang Y, et al. Schimke immuno-osseous dysplasia: SMARCAL1 loss-of-function and phenotypic correlation. J Med Genet. 2009 Jan;46(1):49-59.
36. Hossein Babaei A, Inaloo S, Basiratnia M, Derakhshan A. Early Onset Cerebral Infarction in Schimke Immuno-Osseous Dysplasia. Iran J Child Neurol. 2018 Summer;12(3):126-32.
37. Turro E, Astle WJ, Megy K, Graf S, Greene D, Shamardina O, et al. Whole-genome sequencing of patients with rare diseases in a national health system. Nature. 2020 Jul;583(7814):96-102.
38. Stranneheim H, Lagerstedt-Robinson K, Magnusson M, Kvarnung M, Nilsson D, Lesko N, et al. Integration of whole genome sequencing into a healthcare setting: high diagnostic rates across multiple clinical entities in 3219 rare disease patients. Genome Med. 2021 Mar 17;13(1):40.
39. Hood RL, Lines MA, Nikkel SM, Schwartzentruber J, Beaulieu C, Nowaczyk MJ, et al. Mutations in SRCAP, encoding SNF2-related CREBBP activator protein, cause Floating-Harbor syndrome. Am J Hum Genet. 2012 2/10/2012;90(2):308-13.
40. Reschen M, Kini U, Hood RL, Boycott KM, Hurst J, O'Callaghan CA. Floating-Harbor syndrome and polycystic kidneys associated with SRCAP mutation. Am J Med Genet A. 2012 Dec;158A(12):3196-200.
41. Seifert W, Meinecke P, Kruger G, Rossier E, Heinritz W, Wusthof A, et al. Expanded spectrum of exon 33 and 34 mutations in SRCAP and follow-up in patients with Floating-Harbor syndrome. BMC Med Genet. 2014 Nov 30;15:127.
42. Yagi H, Takagi M, Narumi S, Hasegawa T, Nishimura G, Hasegawa Y. Stippled calcification in an infant with a recurrent SRCAP gene mutation. Am J Med Genet A. 2016 Apr;170A(4):1088-91.

43. Zhang S, Chen S, Qin H, Yuan H, Pi Y, Yang Y, et al. Novel genotypes and phenotypes among Chinese patients with Floating-Harbor syndrome. Orphanet J Rare Dis. 2019 Jun 14;14(1):144.
44. Ko J, Pomerantz JH, Perry H, Shieh JT, Slavotinek AM, Oberoi S, et al. Case Report of Floating-Harbor Syndrome With Bilateral Cleft Lip. Cleft Palate Craniofac J. 2020 Jan;57(1):132-36.
45. Unal S, Alanay Y, Cetin M, Boduroglu K, Utine E, Cormier-Daire V, et al. Striking hematological abnormalities in patients with microcephalic osteodysplastic primordial dwarfism type II (MOPD II): a potential role of pericentrin in hematopoiesis. Pediatr Blood Cancer. 2014 Feb;61(2):302-5.
46. Rauch A, Thiel CT, Schindler D, Wick U, Crow YJ, Ekici AB, et al. Mutations in the pericentrin (PCNT) gene cause primordial dwarfism. Science. 2008 2/8/2008;319(5864):816-19.
47. Xiong HY, Alipanahi B, Lee LJ, Bretschneider H, Merico D, Yuen RK, et al. RNA splicing. The human splicing code reveals new insights into the genetic determinants of disease. Science. 2015 Jan 9;347(6218):1254806.
48. Filonava L, Torres AG, Ribas de Pouplana L. A novel cause for primordial dwarfism revealed: defective tRNA modification. Genome Biol. 2015 Oct 1;16:216.
49. Shaheen R, Abdel-Salam GM, Guy MP, Alomar R, Abdel-Hamid MS, Afifi HH, et al. Mutation in WDR4 impairs tRNA m(7)G46 methylation and causes a distinct form of microcephalic primordial dwarfism. Genome Biol. 2015 Sep 28;16:210.
50. Braun DA, Shril S, Sinha A, Schneider R, Tan W, Ashraf S, et al. Mutations in WDR4 as a new cause of Galloway-Mowat syndrome. Am J Med Genet A. 2018 Nov;176(11):2460-65.
51. Chen X, Gao Y, Yang L, Wu B, Dong X, Liu B, et al. Speech and language delay in a patient with WDR4 mutations. Eur J Med Genet. 2018 Aug;61(8):468-72.
52. Trimouille A, Lasseaux E, Barat P, Deiller C, Drunat S, Rooryck C, et al. Further delineation of the phenotype caused by biallelic variants in the WDR4 gene. Clin Genet. 2018 Feb;93(2):374-77.
53. Baker EK, Brewer CJ, Ferreira L, Schapiro M, Tenney J, Wied HM, et al. Further expansion and confirmation of phenotype in rare loss of YWHAE gene distinct from Miller-Dieker syndrome. Am J Med Genet A. 2023 Feb;191(2):526-39.
54. Denomme-Pichon AS, Collins SC, Bruel AL, Mikhaleva A, Wagner C, Vancollie VE, et al. YWHAE loss of function causes a rare neurodevelopmental disease with brain abnormalities in human and mouse. Genet Med. 2023 Mar 28:100835.
55. Ostergaard JR, Graakjaer J, Brandt C, Birkebaek NH. Further delineation of 17p13.3 microdeletion involving CRK. The effect of growth hormone treatment. Eur J Med Genet. 2012 1/2012;55(1):22-26.

56. Deodati A, Inzaghi E, Germani D, Fausti F, Cianfarani S. Crk Haploinsufficiency Is Associated with Intrauterine Growth Retardation and Severe Postnatal Growth Failure. Horm Res Paediatr. 2021;94(11-12):456-66.
57. Fabre A, Martinez-Vinson C, Roquelaure B, Missirian C, Andre N, Breton A, et al. Novel mutations in TTC37 associated with tricho-hepato-enteric syndrome. Hum Mutat. 2011 Mar;32(3):277-81.
58. Bourgeois P, Esteve C, Chaix C, Beroud C, Levy N, consortium Tc, et al. Tricho-Hepato-Enteric Syndrome mutation update: Mutations spectrum of TTC37 and SKIV2L, clinical analysis and future prospects. Hum Mutat. 2018 Jun;39(6):774-89.
59. Lin YC, Niceta M, Muto V, Vona B, Pagnamenta AT, Maroofian R, et al. SCUBE3 loss-of-function causes a recognizable recessive developmental disorder due to defective bone morphogenetic protein signaling. Am J Hum Genet. 2021 Jan 7;108(1):115-33.
60. Zanoni P, Steindl K, Sengupta D, Joset P, Bahr A, Sticht H, et al. Loss-of-function and missense variants in NSD2 cause decreased methylation activity and are associated with a distinct developmental phenotype. Genet Med. 2021 Aug;23(8):1474-83.
61. Oh RY, Deshwar AR, Marwaha A, Sabha N, Tropak M, Hou H, et al. Biallelic loss-of-function variants in RABGAP1 cause a novel neurodevelopmental syndrome. Genet Med. 2022 Nov;24(11):2399-407.
62. Lin Z, Zhao J, Nitoiu D, Scott CA, Plagnol V, Smith FJ, et al. Loss-of-function mutations in CAST cause peeling skin, leukonychia, acral punctate keratoses, cheilitis, and knuckle pads. Am J Hum Genet. 2015 Mar 5;96(3):440-7.
63. Andrews A, Maharaj A, Cottrell E, Chatterjee S, Shah P, Denvir L, et al. Genetic Characterization of Short Stature Patients With Overlapping Features of Growth Hormone Insensitivity Syndromes. J Clin Endocrinol Metab. 2021 Oct 21;106(11):e4716-e33.
64. Rezende RC, Menezes de Andrade NL, Branco Dantas NC, de Polli Cellin L, Victorino Krepischi AC, Lerario AM, et al. Exome Sequencing Identifies Multiple Genetic Diagnoses in Children with Syndromic Growth Disorders. J Pediatr. 2024 Feb;265:113841.
65. Monies D, Abouelhoda M, Assoum M, Moghrabi N, Rafiullah R, Almontashiri N, et al. Lessons Learned from Large-Scale, First-Tier Clinical Exome Sequencing in a Highly Consanguineous Population. Am J Hum Genet. 2019 Jun 6;104(6):1182-201.
66. Gkourogianni A, Andrew M, Tyzinski L, Crocker M, Douglas J, Dunbar N, et al. Clinical Characterization of Patients With Autosomal Dominant Short Stature due to Aggrecan Mutations. J Clin Endocrinol Metab. 2017 Feb 01;102(2):460-69.

67. Domene HM, Hwa V, Argente J, Wit JM, Camacho-Hubner C, Jasper HG, et al. Human acid-labile subunit deficiency: clinical, endocrine and metabolic consequences. Horm Res. 2009 2009;72(3):129-41.

Figure Legends

Fig. 1: Diagram showing the selection of short patients with consanguineous parents who were investigated and the results.

Figure 1.

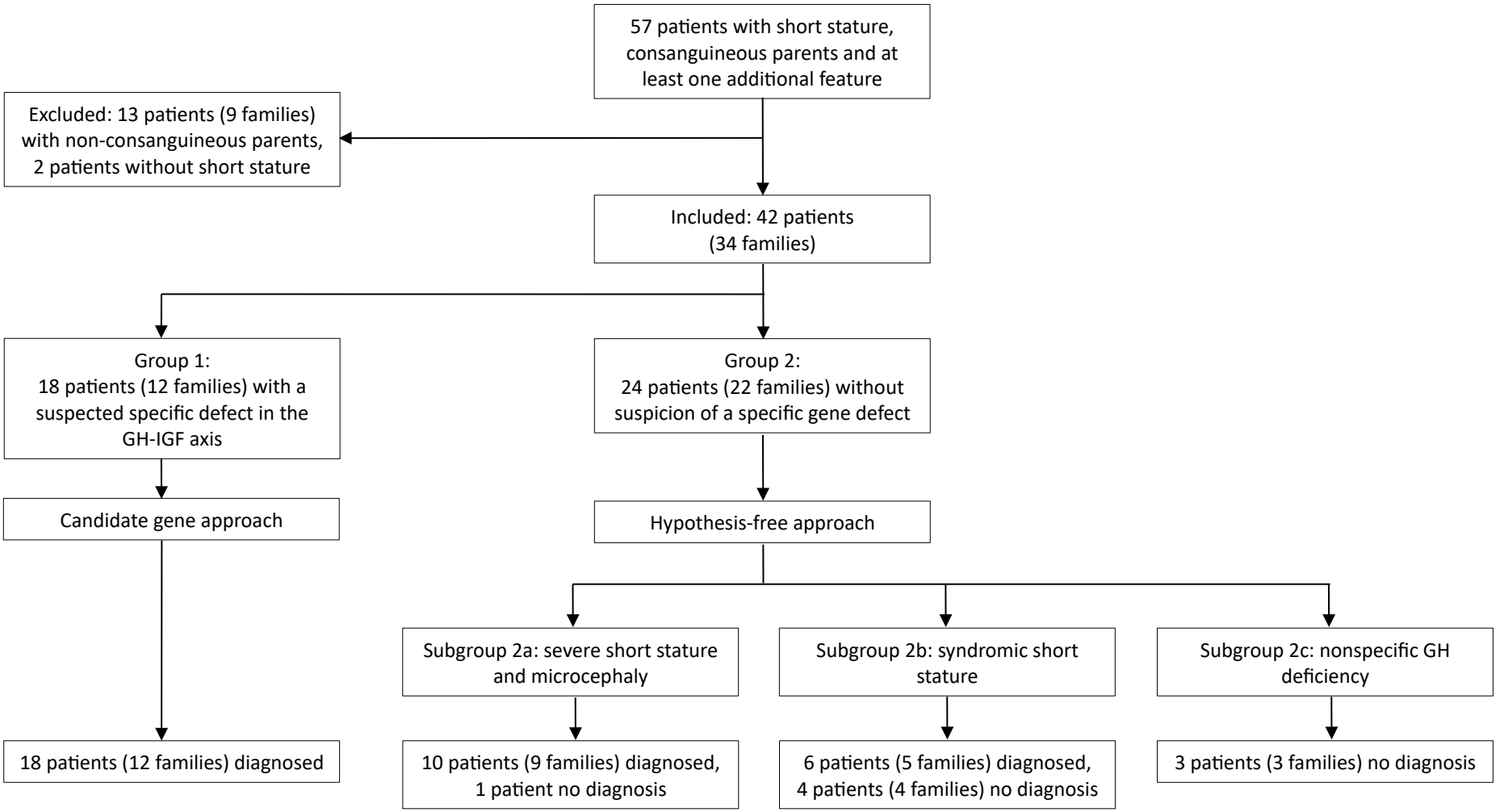
57 patients with short stature, consanguineous parents and at least one additional feature
Excluded: 13 patients (9 families) with non-consanguineous parents, 2 patients without short stature
Included: 42 patients (34 families)
Group 1: 18 patients (12 families) with a suspected specific defect in the GH-IGF axis
Group 2: 24 patients (22 families) without suspicion of a specific gene defect
Candidate gene approach
Hypothesis-free approach
Subgroup 2a: severe short stature and microcephaly
Subgroup 2b: syndromic short stature
Subgroup 2c: nonspecific GH deficiency
18 patients (12 families) diagnosed
10 patients (9 families) diagnosed, 1 patient no diagnosis
6 patients (5 families) diagnosed, 4 patients (4 families) no diagnosis
3 patients (3 families) no diagnosis

Table 1. Group 1; genetic diagnosis in patients suspected of a specific genetic defect in the GH-IGF axis using a candidate gene approach

Family nr	Nr of cases	Gene	Inheri-tance	Genomic variant[2]	cDNA[2]	Protein[2]	Classification *ACMG category*	Diagnosis, MIM nr
1	2	*GH1*	Hom	NC_000017.10:g.(?_61995432)_(61994614_?)del	NM_000515.3: Deletion exon 3, 4, 5	Truncated protein/ No protein expressed	Pathogenic *PSV1, PM3*	IGHD1A, #262400
2	1	*GHR*	Hom	Chr5(GRCh37): g.42695096A>C	NM_000163.4: c.344A>C	p.(Asn115Thr)	Likely Pathogenic *PM1, PM2, PM3_sup, PP5*	Laron syndrome #262500
3	3	*GHR*	Hom	Chr5(GRCh37): g.42695096A>C	NM_000163.4: c.344A>C	p.(Asn115Thr)	Likely Pathogenic *PM1, PM2, PM3_sup, PP5*	Laron syndrome #262500
4	2	*GHR*	Hom	Chr5(GRCh37): g.42718186T>A	NM_000163.4: c.908T>A	p.(Val303Asp)[1]	Likely Pathogenic *PM2, PM3_sup, PP3, PP4_mod*	Laron syndrome #262500
5	2	*GHR*	Hom	Chr5(GRCh37): g.42689028C>T	NM_000163.4: c.173C>T	p.(Ser58Leu)	Likely Pathogenic *PM2, PM3_sup, PP3, PP5*	Laron syndrome #262500
6	1	*GHR*	Hom	Chr5(GRCh37): g.42700896A>G	NM_000163.4: c.618+792A>G	p.?	Likely Pathogenic *PS3, PM2, PM3_sup, PP5*	Laron syndrome #262500
7	1	*STAT5B*	Hom	Chr17(GRCh37): g.40368052del	NM_012448.3: c.1453delG	p.(Asp485Thrfs*29)[1]	Pathogenic *PVS1, PM2, PM3_sup*	GHISID1, #245590
8	2	*IGFALS*	Hom	Chr16(GRCh37): g.1840957C>T	NM_004970.2: c.1462G>A	p.(Asp488Asn)[1]	Likely Pathogenic *PS4, PM2, PM3_sup*	ACLSD #615961
9	1	*IGFALS*	Hom	Chr16(GRCh37): g.1840957C>T	NM_004970.2: c.1462G>A	p.(Asp488Asn)[1]	Likely Pathogenic *PS4, PM2, PM3_sup*	ACLSD #615961
10	1	*IGFALS*	Hom	Chr16(GRCh37): g.1842168T>C	NM_004970.2: c.251A>G	p.(Asn84Ser)[1]	Likely Pathogenic *PS4, PM2, PM3_sup, PP3*	ACLSD #615961
11	1	*IGFALS*	Hom	Chr16(GRCh37): g.1840942del	NM_004970.2: c.1477del	p.(Arg493Alafs*176)[1]	Likely Pathogenic *PVS1, PM2, PM3_sup*	ACLSD #615961
12	1	*IGFALS*	Comp Het	Chr16(GRCh37): g.1842168T>C g.1840957C>T	NM_004970.2: c.251A>G c.1462G>A	p.(Asn84Ser)[1] p.(Asp488Asn)[1]	Likely Pathogenic (both) *PM1, PM2, PM3, PP3*	ACLSD #615961

[1] Novel variant

[2] Human genome variation society (HGVS) nomenclature

Abbreviations: ACMG, American College of Medical Genetics and Genomics; Comp Het, compound heterozygous; Gr, Group; Het, heterozygous; Hom, homozygous; LP, likely pathogenic; P, Pathogenic; nr, number; ACLSD, acid-labile subunit deficiency; GHDP, isolated partial growth hormone deficiency; GHISID1, growth hormone insensitivity syndrome with immune dysregulation 1, autosomal recessive; IGHD1A, isolated growth hormone deficiency, type Ia

Table 2. Genetic diagnosis in patients with microcephalic severe short stature (group 2a) and syndromic short stature (group 2b) using a hypothesis-free approach

Family (#cases)	Gene	Inheri-tance	Genomic variant (HGVS)	cDNA	Protein	ACMG category	Diagnosis, MIM
Group 2a							
13	*SMARCAL1*	Hom	Chr2(GRCh37): g.217341863G>A	NM_014140.4: c.2459G>A	p.(Arg820His)	Pathogenic *PP5 PM2 PM3 PP3*	SIOD #242900
14	*SMARCAL1*	Hom	Chr2(GRCh37): g.217341863G>A	NM_014140.4: c.2459G>A	p.(Arg820His)	Pathogenic *PP5 PM2 PM3 PP3*	SIOD, #242900
15	*SRCAP*	Het (*de novo*)	Chr16(GRCh37): g.30748664C>T	NM_006662.3: c.7303C>T	p.(Arg2435*)	Pathogenic *PP5 PVS1 PM2 PS2*	Floating-Harbor #136140
16	*PCNT*	Hom	Chr21(GRCh37): g.47831167dup	NM_006031.6: c.5180dup	p.(Asn1727Lysfs*14)[1]	Likely pathogenic *PVS1 PM2*	MOPD2 #210720
17 (2)	*PCNT*	Hom	Chr21(GRCh37): g.47786998G>T	NM_006031.6: c.3109G>T	p.(Glu1037*)	Pathogenic *PVS1 PM2 PP5 PM3*	MOPD2 #210720
18	*PCNT*	Hom	Chr21(GRCh37): g.47766655A>G	NM_006031.6: c.721-2A>G	p.?	Likely pathogenic *PVS1 PM2*	MOPD2 #210720
19	*PCNT*	Hom	Chr21(GRCh37): g.47809114_47809346del	NM_006031.6: c.3608_3840del	p.(Pro1204Glyfs*11)[1]	Likely pathogenic *1A 2B 2E 3A*	MOPD2 #210720
20	*WDR4*	Hom	Chr21(GRCh37): g.44283575C>T	NM_018669.6: c.428G>A	p.(Gly143Glu)[1]	Likely pathogenic *PM2 PM3_SUP PM1 PP3_SUP*	MIGSB #618346
21	*GHSR*	Het (mat)	Chr3(GRCh37): g.172163003G>C	NM_198407.2: c.1049C>G	p.(Thr350Ser)	VUS *PM2 PP1 PP5 BP4*	GHDP #615925
Group 2b							
23	two 17p13.3 deletions	Het[a] (probably *de novo*)	arr[hg19] 17p13.3(525-1,922,715)x1, 17p13.3(2,024,217-2,262,703)x1[1]				
24	*TTC37*	Hom	Chr5(GRCh37): g.94803618C>T	NM_014639.4:c.4572G>A	p.(Trp1524*)	Likely pathogenic *PM2 PVS1 PP5*	THES1 #222470
25 (2)	*SCUBE3*	Hom	Chr6(GRCh37): g.35213204T>C	NM_152753.4: c.2599+2T>C	p.?[1]	Likely pathogenic *PVS1 PM2 PM3*	SSFSC2 #619184

26	*NSD2*	Het (*de novo*)	Chr4(GRCh37): g.1905987dup	NM_001042424.3: c.642dup	p.(Asp215Argfs*10)[1]	Pathogenic *PVS1 PS2 PM2*	WHS #194190
27	*RABGAP1*	Hom	Chr9(GRCh37): g.125861049dup	NM_012197.4: c.2789dup	p.(Asn930Lysfs*7)[1]	Likely pathogenic *PVS1 PM2 M3_SUP*	*RABGAP1*-related syndrome

[1]Novel variant. [a]The deletions were not found in the mother's DNA, but father's DNA was not available.
Abbreviations: ACMG, American College of Medical Genetics and Genomics; Comp Het, compound heterozygous; Gr, Group; HGVS, human genome variation society; Het, heterozygous; Hom, homozygous; LP, likely pathogenic; P, Pathogenic; syndr, syndrome; GHDP, isolated partial growth hormone deficiency; MDLS, Miller-Dieker Lissencephaly Syndrome; MIGSB, Microcephaly, Growth Deficiency, Seizures, And Brain Malformations; MOPD2, Microcephalic Osteodysplastic Primordial Dwarfism Type 2; SIOD, Schimke Immunoosseous Dysplasia; SSFSC2, Short Stature, Facial Dysmorphism, And Skeletal Anomalies With Or Without Cardiac Anomalies 2; SSOAOD, Short Stature And Advanced Bone Age, With Or Without Early-Onset Osteoarthritis And/Or Osteochondritis Dissecans
THES1, Trichohepatoenteric Syndrome 1; WHS, Wolf-Hirschhorn Syndrome

Supplemental Data

Genetic findings in short Turkish children born to consanguineous parents

Sjoerd D. Joustra*, Emregul Isik*, Jan M. Wit*, Gonul Catli, Ahmet Anik, Belma Haliloglu, Nurgun Kandemir, Elif Ozsu, Yvonne M.C. Hendriks, Christiaan de Bruin, Sarina G. Kant, Angel Campos-Barros, Rachel C. Challis, David Parry, Margaret E. Harley, Andrew Jackson, Monique Losekoot[#], Hermine A. van Duyvenvoorde[#]

*These authors contributed equally to this paper

#These authors share last authorship

SUPPLEMENTAL LABORATORY INFORMATION

In the department of Clinical Genetics in Leiden, genomic DNA was extracted from blood samples using the Chemagen automated DNA isolation work station (Janus Chemagic 360, Perkin Elmer). Analysis of candidate genes was performed using Sanger sequencing of the exons including +/- 20 nucleotides of intronic sequences and analysis of intron 6 of *GHR* was performed using standard procedures. MLPA kits P216 and P262 MRC Holland, The Netherlands were used for CNV detection. WES was performed using the Agilent SureSelectXT Human All Exome V5 or V7 kit following the manufacturer instructions in an Illumina platform, with >94% of targeted regions were covered at >= 20x depth. The exome sequencing protocol was validated for clinical use according to ISO 15189. After 'read alignment' using BWA and 'variant calling' using GATK, the annotation was performed using the in-house sequence analysis pipeline 'Modular GATK-Based Variant Calling Pipeline (MAGPIE)'. MOON software (Invitae) was used for further HPO-based (https://hpo.jax.org/app/) analysis and interpretation of variants. The pathogenicity of variants was classified according to the ACMG/AMP criteria [1, 2]. A full list of detected variants in our patients is available on request.

In the MRC HGU in Edinburgh (United Kingdom), for patients 14-20 and 22, genomic DNA was extracted from blood samples by standard methods or from saliva samples using Oragene collection kits according to the manufacturer's instructions. Informed consent was obtained from all participating families as a research study approved by the Scottish Multicentre Research Ethics Committee (05/MRE00/74). Mutation analysis of *PCNT* was performed by Sanger sequencing of all coding exons [3], in cases phenotypically matching MOPD II. For remaining cases, patients were screened for Microcephaly and Primordial dwarfism genes using a next generation sequencing panel. Target enrichment was performed using a custom-designed Twist baits (Twist Bioscience), with coverage of >99% of all coding exons as reported on RefSeq and their immediate intronic sequences (+/- 15bp) for 91 genes. Library preparation was performed with Nextera Flex for Enrichment reagent

kit according to manufacturer's instructions (Illumina), with 150bp paired-end sequencing on the MiSeq platform (Illumina), with >98% of targeted regions were covered at >= 20x depth. The following genes associated with primordial dwarfism, primary microcephaly and associated syndromes were analysed: *ANKLE2, ANKRD11, ARCN1, ASPM, ASXL3, ATR, ATRIP, ATRX, BLM, CASK, CDC45, CDC6, CDK5RAP2, CDKL5, CDKN1C, CDT1, CENPE, CENPF, CENPJ, CEP135, CEP152, CEP63, CIT, CREBBP, DDX11, DNA2, DNMT3A, DONSON, DPP6, DYRK1A, EED, EP300, ERCC6, ERCC8, ESCO2, FANCD2, FOXG1, GMNN, IGF1, IGF1R, KAT6B, KIF11, KMT2A, KNL1, LARP7, LIG4, MCM4, MCPH1, MED12, MRE11A, MYCN, NBN, NCAPD2, NCAPD3, NCAPH, NDE1, NSUN2, ORC1, ORC4, ORC6, PCNT, PHC1, PHGDH, PIK3R1, PLK4, PNKP, POC1A, POLA1, POLE, RAD50, RAD51, RBBP8, RBM10, RNU4ATAC, RTTN, SETD2, SMARCAL1, SRCAP, STIL, TCF4, TONSL, TOP3A, TRAIP, TUBGCP6, VPS13B, WDR4, WDR62, WHSC1, XRCC1, XRCC4, ZNF335*.

In the INGEMM in Madrid (Spain), for patients 32-34, WES of the genomic DNA of the three probands and available parent samples was performed using the Agilent SureSelect Human All Exome V6 (58M) kit following the manufacturer instructions in an Illumina Novaseq 6000 platform. Q30 score (i.e inferred base call accuracy = 99.9%) was > 80%, average depth 178.5x (range: 135.7-293.3), and average bp % with coverage >20x: 97.13% (range: 96-98.5%). Variants listed in the VCF file were filtered and prioritized based on sequence quality assessment (Q>30; mean coverage >90x; % of bp with coverage>20x >80%); population frequency (minor allele frequency <1% in gnomAD (V2.1.1); variant effect (missense, nonsense, frameshift, splicing effect), in silico pathogenicity prediction (CADD_1.6 score >20; [4], and inheritance pattern, with help of the software package (VarSeq V2.5.1, Golden Helix, MT, USA). BAM files were visualized with help of the Alamut Visual Plus software (V1.6.1, Sophia Genetics SA, Switzerland). Genic and intragenic CNVs were screened with the bioinformatics tool VarSeq CNV Caller (Iacocca et al., 2017), included in the VarSeq (V2.5.1) software (Golden Helix, MT, USA). Variant classification was performed according to ACMG recommendations [1]. A full list of detected variants in our patients is available on request.

SUPPLEMENTARY CLINICAL INFORMATION

For all patients, numerical data are presented in **Supplementary Tables 1-4**. For group 2 (subgroups 2a, 2b and 2c), clinical data are presented in the following paragraphs.

Subgroup 2a: subjects with severe short stature (height <-3.5 SDS) and microcephaly (head circumference <-3.0 SDS) (Supplementary Table S2)

Patients 13 and 14 from two reportedly unrelated families living in the same city (Gaziantep, Turkey) were homozygous for an identical pathogenic variant in *SMARCAL1*, consistent with the diagnosis of Schimke immuno-osseous dysplasia (MIM #242900). Most of their clinical features were similar as

previously reported [5], such as the coarse or fine hair, depressed nasal bridge and bulbous nasal tip, short neck, hypertension, proteinuria, lumbar lordosis and protruding abdomen. Case 13 passed away at 8 years, and his sister with the same clinical presentation (nephrotic syndrome) had died of severe pneumonia at 8 years. Case 14 is on peritoneal dialysis since the age of 4 years. At 9.6 years of age, she suffered from hypertension, numbness in hands and feet, and seizures due to recurrent cerebral strokes. Her height and weight SDS had further decreased (-9.0 and -7.1 SDS, respectively). One of the patient's cousins was also diagnosed with Schimke immuneosseous dysplasia and died from renal insufficiency and cerebral stroke at 12 years of age. We observed several clinical features in these patients that were not included in the clinical synopsis of the syndrome in OMIM: preterm birth (32 and 28 weeks, respectively); microcephaly (head circumference -3.7 SDS); and serum IGF-I close to the lower limit of the reference range (-1.7 SDS). In contrast to previous observations, body proportions as assessed by sitting height/height ratio were normal (0.6 and -1.4 SDS, respectively). Radiological images of pelvic bones showed bilateral displayed hypoplastic capital femoral epiphyses, without other radiological abnormalities reported in patients with Schimke immuno-osseous dysplasia.

In patient 15 a heterozygous pathogenic *SRCAP* variant was detected, consistent with Floating-Harbor syndrome (MIM #136140). He shared many of the typical clinical characteristics that were described previously for children with this syndrome, including prenatal onset of short stature, triangular face, posteriorly rotated ears, prominent nose, wide columella, smooth philtrum, thin lips, broad mouth, and short neck. Head circumference was initially very low (-3.7 SDS), in line with a few previous reports, including one from Turkey [6, 7]. At follow-up at 7.9 years, height SDS remained -3.7 SDS, but head circumference SDS had increased to -2.4. The patient had a severe speech delay (spoke only ≈50 words, no full sentences). Serum IGF-I was normal. The same variant has been found in six previous reports [8-13].

In 5 patients from families 16-19, homozygosity was shown for four different pathogenic *PCNT* variants (3 of which are novel), with the typical clinical features of microcephalic osteodysplastic primordial dwarfism type 2 (MOPD2, MIM #210720). Case 16 has persistent anaemia and iron deficiency. We have not detected persistent leucocytosis or thrombocytosis in any of these patients.

In patient 20 a novel homozygous *WDR4* variant was detected, associated with a recently reported form of primordial dwarfism, called "microcephaly, growth deficiency, seizures, and brain malformations" (MIGSB, MIM #618346). The clinical features of our patient included a low birth size, seizures from 3 years of age and a severely delayed speech and psychomotor development (she started walking at 5 years). An MRI showed diffuse cerebellar atrophy and small focal signal changes at bilateral frontal subcortical white matter and centrum semi-ovale. These features are similar to previous reports [14-17]. The pituitary had a height of 6.5 mm and volume of 300 mm^3 (reference

198.92±61.80 mm^3) [18] at the age of 10.8 years. Serum TSH was 9.77 mU/L, but FT4, FT3, ACTH, cortisol and prolactin were normal. Levothyroxine treatment was started. Interestingly, this patient also showed an extremely low serum IGF-I and a low GH peak (3.68 ng/mL) in a stimulation test (**Supplementary Table S2**).

In patient 21 we found a maternally inherited heterozygous variant of unknown significance (VUS) in *GHSR* in the proband and three short relatives: the mother (-3.3 SDS), a maternal aunt (-2.7 SDS) and one of the proband's sisters (-2.2 SDS). The variant was not present in relatives with normal stature. The proband's father has a normal height (-1.0 SDS). The patient presented at 11.8 years with non-dysmorphic short stature (-3.7 SDS), developmental delay and intellectual deficit. At 14.9 years (Tanner stage 3), height SDS had decreased to -4.1 SDS and serum IGF-I and IGFBP-3 had decreased, though the peak GH in a stimulation test was normal. RhGH treatment was administered for 9 months, resulting in a height increment of 9.5 cm and height SDS of -3.4 SDS, while bone age was 2.7 years delayed. Reported adult height was 165 cm (-2.0 SDS). The immature Tanner stage at 14.9 years and the subsequent increase of height in late adolescence from -4.1 to -2.0 is strongly suggestive for delayed puberty. We speculate that the combination of short stature in childhood and delayed puberty in the proband is caused by the maternal *GHSR* variant. Since this variant cannot explain the patient's microcephaly and intellectual deficit, we expanded the DNA search to these features, and found two novel heterozygous missense variants in *CCND2*, located near each other on the same allele (in cis). Based on the recently reported five cases from three families with a heterozygous loss-of-function *CCND2* variant in the proximal region of the gene, the main effect of such variant is microcephaly, mildly simplified cortical gyral pattern, symmetric borderline short stature, and mild developmental delay [19]. Segregation analysis showed that this variant was also carried by the father. Unfortunately, information on paternal head circumference and intelligence is unavailable. Functional studies are needed to test the hypothesis that the paternal *CCDN2* variants are associated with the patient's microcephaly and developmental delay.

In patient 22 no genetic cause could be found. He was born small for gestational age (SGA) and presented with severe short stature, microcephaly, developmental delay, mild dysmorphic features (thick eyebrows, prominent eyelashes, short philtrum with low hanging columella, narrow mouth with rather thick lips) and borderline low IGF-I, IGFBP-3 and GH peak. His first-degree consanguineous parents were borderline short (height -2.1 and -1.4 SDS, respectively). Brain MRI showed a small pituitary (160 mm^3, reference range 300±100 mm^3). The patient was treated with rhGH but was then lost to follow up. No pathogenic variants were identified in a targeted gene panel nor in microarray analysis.

Subgroup 2b: Supplementary clinical information on subjects with syndromic short stature (Supplementary Table S3)

In DNA from patient 23, two 17p13.3 microdeletions separated by a small non-deleted region of 101.5 kb were found. The relatively large distal deletion contains *YWHAE*, *CRK* and 29 other genes, but neither deletion contains *PAFAH1B1*. Similar genetic findings have been reported in 19 individuals with similar clinical features as our patient [20]. The distinctive facial dysmorphism and malformations of cortical development appear to be associated with the deletion of *YWHAE*, but these features are more severe in patients with a larger deletion [21]. Our patient was born extremely SGA (weight -6.1 SDS, length -3.9 SDS), as the oldest of two children of first-degree consanguineous parents. Father's height was unknown, maternal height was 155 cm (-1.4 SDS). Psychomotor milestones were normal. She presented at 2.9 years with severe short stature, depigmented and sparse hair, frontal bossing and relative macrocephaly and increased sitting height to height ratio (4.0 SDS) associated with rhizomelia. Serum IGF-I and IGFBP-3 were strongly decreased, but GH secretion was normal. Cardiac sonography showed a secundum atrial septum defect and patent ductus arteriosus.

Genetic analysis for patient 24 showed a homozygous pathogenic *TTC37* variant, consistent with Trichohepatoenteric syndrome 1 (THES1, MIM 222470). He presented at 4.2 years with a history of pneumonia at 3 months, frequent diarrhoea until 3 years, lactose intolerance and eczema. Diarrhoea had not responded to the lactose-free diet. Stool frequency was acceptable on a peptide-based formula. We observed 6 renal stones in the right kidney (7 mm diameter). He is the first child of first-degree consanguineous parents (father's height -1.4 SDS, mother's height -1.3 SDS). At physical examination, microcephaly, brittle nails and dry discoloured woolly hair were noted. Sitting height/height ratio was 1.3 SDS. Several clinical features of the patient have been associated with the broad spectrum of phenotypic expression in THES1, such as intrauterine growth retardation, woolly hair, facial dysmorphism, intractable diarrhoea in infancy and immunodepression [22, 23]. Microcephaly was noted in a recently reported case [24]. Complete blood count, immunoglobulins and antibody responses were normal, but the CD4 percentage was decreased.

The two siblings in family 25 were included in a paper on 9 families with a novel dysmorphic short stature syndrome (MIM #619184) caused by bi-allelic *SCUBE3* variants. For details regarding clinical information the reader is referred to this publication [25].

In patient 26 we detected a *de novo* heterozygous frameshift variant in *NSD2*. She was born severely small for gestational age. At presentation at the clinic, she was short and microcephalic, and showed mild dysmorphic features (triangular face, periorbital hyperpigmentation, short philtrum and thin elevated nasal bridge). Both parents had a borderline low height (-1.9 SDS). IGF-I was normal. Key features of this syndrome are pre- and postnatal growth delay, failure to thrive, microcephaly,

distinctive facial features and normal IGF-I, all consistent with observations in our patient. Most patients display mild cognitive impairment, but some go to a regular school as did our patient. Formal IQ testing was not performed in our patient.

In patient 27 we found a novel homozygous variant in *RABGAP1*, which was recently described as the cause of a neurodevelopmental syndrome in five patients (carrying three different variants) with intellectual disability, microcephaly, bilateral sensorineural hearing loss, seizures and overlapping dysmorphic features [26]. In this publication, two out of five patients were short (-2.0 and -3.0 SDS) and the other three had a height in the lower half of the reference range (-0.4, -0.7 and -0.9 SDS). Since mTOR signalling was downregulated in cells of patients with loss-of-function *RAGGAP1* variants [26], and mTORC1 is a key regulator of cell growth and proliferation and mRNA translation, we postulate that short stature is a (non-obligatory) feature of this novel syndrome. The patient was born with a very low birth weight and presented with psychomotor and speech delay, intellectual deficit and bilateral hearing loss. He first started to walk at 26 months. Parents were healthy and a normal height. Presently, he cannot make sentences and cannot make himself understood. He has not had seizures. Height SDS was similarly decreased as head circumference SDS. Dysmorphic features include a coarse face, short neck, mild upslant of palpebral fissures, prominent eyelashes, upsweep of the lateral part of the eyebrows, wide nasal ridge, mildly low-set ears with overfolded upper part of the helix and underfolded lower part, everted lower lip, short hallux on the left side. Bone age was delayed. Serum IGF-I and IGFBP-3 were in the upper half of the reference range (1.3 SDS) and the GH peak in a stimulation test was normal.

In patient 28, no certain clinical or genetic diagnosis could be made. Information on birth size was missing. Besides short stature, there were no complaints. Physical examination showed normal body proportions and no dysmorphic features (head circumference was not measured). The father was severely short (-3.4 SDS) and mother's height was in the lower half of the reference range (-1.6 SDS). All three siblings of the patient were short (range -3.9 to -2.9 SDS). Bone age was 1.5 years delayed. Serum IGF-I and IGFBP-3 were low, but the GH response to a stimulation test was normal. A high dose IGF generation test (100 mcg/kg) revealed a normal IGF-I and IGFBP3 response. A SNP-array showed an interstitial duplication of minimally 618.4 kb, containing a large part of one protein coding gene (*SORCS3*), not previously described [arr[hg19], 10q25.1(106,360,639-106,979,017)x3 paternal]. The SNP-array was not performed in the siblings of this patient. Interestingly, we found a homozygous novel VUS in *IHH* NM_002181.4(IHH):c.877A>G p.(Thr293Ala), and both parents were heterozygous carriers of the variant. Homozygous or compound heterozygous pathogenic variants in the IHH gene are associated with Acrocapitofemoral dysplasia (ACFD, (MIM #600726)), a skeletal dysplasia characterized by postnatal-onset disproportionate short stature, relatively large head, narrow thorax, lumbar lordosis, short limbs, and brachydactyly with small broad nails. In-silico

prediction of pathogenicity of the identified missense variant is not strong, however this variant is not present in homozygous state and only observed in two alleles in heterozygous state in 278,838 control alleles (GnomAD v2.1.1 database). Additional segregation analysis in three siblings with growth failure showed that the *IHH* variant was present in heterozygous (n=2) and homozygous (n=1) state, while the heights of the siblings were similar (range -2.9 – 3.9 SDS). Based on the in-silico prediction and segregation data we hypothesize that the *IHH* variant does not explain the short stature in this family.

In case 29 we detected a paternally transmitted heterozygous variant of uncertain significance (VUS, PM1, PM2 PP3) in *ACAN* (Chr15(GRCh37): g.89386688A>G, NM_001369268.1:c.860A>G p.Tyr287Cys)), the variant turned out to be present in homozygous state in the father. The more severe short stature in the *ACAN* heterozygous patient (-4.0 SDS) compared with her *ACAN* homozygous father (-2.4 SDS), as well as the presence of multiple other clinical features, are suggestive for another genetic defect that is more consistent with the phenotype. The patient was born SGA as the first of four children from first-degree consanguineous parents. Father's height is 161.5 cm (-2.4 SDS) and mother's height 160 cm (-0.5 SDS). No sign of an *ACAN*-related disorder was noticed at visual inspection of the father. Several maternal cousins of the patient are known with short stature with a similar phenotype. The medical history included frequent diarrhoea and otitis in infancy. Psychomotor milestones were normal, but school performance was poor. At physical examination, a broad nasal bridge, retrognathia, mid-facial hypoplasia, short neck, hyperlordosis and a congenital missing fifth toe at the right foot were noted. Body proportions were normal (sitting height/height ratio 0.1 SDS). Serum IGF-I was low but GHD was excluded by a normal GH peak at a GH stimulation test. Radiographic images showed lumbar lordosis and growth arrest lines at the femur and proximal metaphysis of tibia, mild tibial bowing, and gyral impressions at the skull. At 17.7 years she had reached an adult height of 132 cm (-5.3 SDS) with a low BMI (-3.7 SDS). Menarche had occurred at 15 years of age and was followed by oligomenorrhoea. At endocrine testing, serum LH and FSH were normal, as well as other endocrine functions tests. Pelvic ultrasonography showed no abnormality. She is currently on combined oestrogen and progesterone treatment.

In patient 30, no clinical or genetic diagnosis could be made. The patient's past medical history included a very low birth weight, psychomotor and speech delay, intellectual deficit, recurrent otitis media and bilateral hearing loss. At physical examination, she was extremely short with normal body proportions and borderline microcephaly, thus relative macrocephaly. Dysmorphic features included almond eyes, retrognathia, prominent nose and a thin upper lip. She did not show pubertal signs and bone age was delayed. Biochemical screening showed a low serum IGF-I but normal GH peak in a stimulation test. Genetic testing showed that the patient carried a heterozygous pathogenic variant

in *LARP7* (NM_016648.4(LARP7):c.475_478dup, p.Lys160Ilefs*2), but this variant was also present in the normal statured mother (height 0.5 SDS).

In patient 31, no clinical or genetic diagnosis could be made. Past medical history included a low birth weight, bilateral hearing loss (noticed at 1 year of age), delayed psychomotor and speech development and subnormal cognitive development. A hearing device was administered at 3.4 years. He was short and mildly microcephalic. Dysmorphic features included mildly triangular face, bushy eyebrows, low hanging columella, low-set ears, and mild micrognathia. There was a severe hyperlordosis. Parents are healthy and of normal height. Serum IGF-I and IGFBP-3 were within the reference range. A novel homozygous variant of unknown significance in *SPATA5* was found: Chr4(GRCh37):g.123850301A>G, NM_145207.3: c.395A>G p.(Gln132Arg) (ACMG: PM1, PM2, BP4). The patient presented with several clinical features that seem consistent with "neurodevelopmental disorder with hearing loss, seizures, and brain abnormalities" (NEDHSB, MIM #616577), caused by bi-allelic *SPATA5* variants. These features include short stature, developmental delay, microcephaly, sensorineural hearing loss, low birth size, low set ears and broad/thick eyebrows. Several clinical features of the patient, including poor growth and facial Gestalt, are similar to the cases 2 and 3 reported by Kurata et al [27] and a patient published by Buchert et al [28]. However, the results of the *in silico* prediction tools of the *SPATA5* variant leaves us in doubt whether this genetic finding is associated with the clinical presentation. Functional studies of this variant are needed to confirm our speculation.

Subgroup 2c: Subjects with growth hormone deficiency

Numerical data of the 3 patients with GHD are shown in **Supplementary Table 4**. The results of the WES analysis were non-conclusive.

Patient 32 was born with a normal birth weight and besides severe proportionate short stature there were no complaints. Head circumference was in the lower half of the reference range. Paternal and maternal heights were -2.1 and -2.8 SDS. Serum IGF-I and IGFBP-3 were low. The GH peaks in two stimulation tests was were 3.3 ng/mL (clonidine) and 6.8 ng/mL (glucagon). An IGF generation test showed a positive response. Other pituitary hormones were normal and a brain MRI showed a normal pituitary. Candidate gene testing for *GH1, GHRHR* and *GHSR* showed normal results, and WES results were inconclusive. RhGH treatment was started at 6 years of age. On treatment, the first-year height velocity was 7.5 cm, but compliance appeared poor. The patient was then lost to follow up.

Patient 33 was born with a borderline low weight. The medical history was uneventful. Height was short, head circumference normal, and sitting height/height ratio elevated (3.2 SDS). No dysmorphic features were noted. Paternal and maternal heights were -2.1 and -1.5 SDS. Serum IGF-I was in the lowest half of the reference range, but serum IGFBP-3 was very low. The GH peaks in stimulation

tests were 0.6 ng/mL (glucagon) and 2.2 ng/mL (clonidine). The brain MRI was normal. Trio WES analysis did not yield a positive result.

Patient 34 was born with a normal weight. The medical history was uneventful except for widespread severe eczema, cheilitis, peeling skin, onychomycosis and acral punctate keratosis starting at 1 year of age. Height was proportionately short and head circumference was borderline low. Besides his skin phenotype, physical examination showed curly hair, a relatively small face, and coarse facial features. Paternal and maternal heights are -0.8 and -1.7 SDS. Serum IGF-I and IGFBP-3 were low and the GH peak in stimulation tests were 0.2 ng/mL (glucagon) and 5.5 ng/mL (clonidine). MRI showed a small adenohypophysis, small pituitary stalk and ectopic neurohypophysis. The clinical features suggested Netherton syndrome, but no pathogenic variant was found in *SPINK5*. However, a homozygous likely pathogenic nonsense variant in *CAST* exon 10: Chr5(GRCh38):g.96740074A>T, NM_001042440.5: c.712A>T p.(Lys238*), (ACMG: PVS1, PM2) was found by WES analysis of the proband and his mother [29], which we consider to be the cause of his skin phenotype.

291 Supplementary Table S1. Group 1; Clinical and biochemical features and candidate gene analysis in subjects with pathogenic variants in the GH-IGF-I axis

292

Patient number	**Sex**	**Age (yrs)**	**BA delay (yrs)**	**BW SDS**	**Height SDS**	**HC SDS**	**BMI SDS**	**IGF-I SDS**	**IGFBP-3 SDS**	**GH basal (ng/mL)**	**GH max (ng/mL)**	**IGF gen test (ng/mL)**	**Clinical features**	**Genetic findins (all homozygous)**
														GH1 gene
1a	M	4.5	1.75	1.1	-4.6	-1.3	-4.6	<-3.0	<-3.0	<0.05	<0.05	nd	Classical, hypoglycaemia	Deletion of *GH1* exon 3, 4 and 5[b]
1b	F	3.2	1.66	2.0	-8.5	-4.0	-0.8	<-3.0	<-3.0	<0.05	<0.05	nd	Classical, hypoglycaemia	
														GHR gene:
2	M	10.1	2.2	-0.2	-4.3	-1.1	-0.7	<-2.8	<-2.6	1.8-1.9	20.0	No resp	Dry skin, orchidopexy	c.344A>C p.Asn115Thr
3a	F	5.0	2.0	0.5	-3.8	-1.6	-0.4	-2.2	-2.8	13.3	nd	+25	Classical	c.344A>C p.Asn115Thr
3b	F	10.7	na	na	-2.4	-2.5	-0.7	-2.6	-3.1	0.7	nd	nd	Classical	
3c	M	8.5	1.5	na	-3.3	-1.5	1.0	-3.2	-3.7	0.8	nd	nd	Classical	
4a	M	7.9	4.3	-1.2	-3.9	-2.0	-1.7	<-3.9	-3.0	1.5	12.1	No resp	Classical	c.908T>A p.Val303Asp
4b	M	4.0	1.5	-1.4	-2.1	-1.6	-1.0	-2.9	-1	1.4	nd	nd	Classical	
5a	F	6.5	3.75	0.3	-7.2	na	0.9	<-3.0	<-3.0	40	nd	No resp	Classical	c.173C>T p.Ser58Leu
5b	F	16.5	6.0	-0.2	-11.2	na	-1.4	<-3.0	<-3.0	3.3, 8.7	40	No resp	Classical	
6	M	2.6	na	-0.8	-3.9	na	0.7	<-3.0	<-3.0	na	17.7	No resp	Hypothyroidism, sensorineural deafness	c.618+792A>G
														STAT5B gene:

7	F	17.6	5.2 (at 15.2)	-1.6	-5.2	-2.0	1.9	undet	<-3.0	0.7	3.8	nd	Eczema, midline hypoplasia, frontal bossing, obesity, PRL↑	c.1453delG p.Asp485Thrfs*29
														IGFALS gene:
8a (A.III.5[a])	M	8.0	2.4	-1.3	-3.7	-2.9	-0.3	-4.5	-9.1	3.8	nd	No resp	none	c.1462G>A p.Asp488Asn
8b (A.III.14[a])	M	11.5	0.4	na	-3.7	-2.8	-0.5	-3.6	-10.2	11.9	12.9	+29	none	
9 (B.IV.2[a])	M	9.4	3.0	-2.6	-2.0	-2.0	-1.2	-3.3	-11.2	0.6	26.4	nd	none	c.1462G>A p.Asp488Asn
10 (C.V.5[a])	F	13.5	2.5	-3.8	-3.6	-2.1	-1.1	-3.2	-8.1	0.4	8.2	nd	none	c.251A>G p.Asn84Ser
11 (D.IV.3[a])	M	11.4	2.0	na	-2.3	-2.2	-1.6	-3.8	-10.0	na	10.3	No resp	none	c.1477del p.Arg493Alafs*176
12 (E.IV.2[a])	F	11.8	-0.5	-2.4	-3.7	-1.9	-0.1	-3.0	-6.5	2,1	12	+21	none	c.251A>G, p.Asn84Ser/ c.1462G>A, p.Asp488Asn

Abbreviations: BA, bone age, BW, birth weight;. hc: head circumference; IGF gen test, IGF generation test; na, not available; Nd, not done; PRL, prolactin; resp, response; undet, undetectable; [a]codes as published in Isik et al [30]. [b]GH1 NC_000017.10:g.(?_61995432)_(61994614 _?)del, a deletion of *GH1* exon 3, 4 and 5 detected with a MLPA assay (MRC-Holland kit P216) where the nucleotide positions g.61995433 and g.61994615 are defined by the first nucleotide of the probe 3'- of the ligation site for respectively exon 3 and exon 5.

Supplementary Table S2. Subgroup 2a; Clinical and biochemical features and genetic findings in subjects with microcephaly (head circumference <-3.0 SDS) and severe short stature (height <-3.5 SDS)

Patient number	Sex	Age (yrs)	BA delay (yrs)	BW SDS	Height SDS	HC SDS	BMI SDS	IGF-1 SDS	IGFBP-3 SDS	GH basal (ng/mL)	GH max (ng/mL)	IGF gen test (ng/mL)	Clinical features	Genetic findings
13	M	5.9	2.3	-4.1	-6.7	-3.7	0.0	-1.7	-4.4	8.0	13.4	nd	Abnormal hair, depressed nasal bridge, bulbous nasal tip, lumbar lordosis, proteinuria, hypertension. Deceased at 8 yrs.	Hom *SMARCAL1* variant, NM_014140.4: c.2459G>A p.Arg820His
14	F	1.9	0.5	-0.4	-5.2	-3.7	-1.3	-3.1	-0.6	2.0	5.6	nd	Fine hair, thin upper lip, bulbous nasal tip, lumbar lordosis, FSGS, end stage renal disease, developmental delay, shallow acetabular fossa	Hom *SMARCAL1* variant, NM_014140.4: c.2459G>A p.Arg820His
15	M	0.9 7.9	na	-2.0	-3.7 -3.7	-3.7 -2.4	-2.5 -0.4	0.1	0.6	13.7	nd	nd	Triangular face, thin and horizontal lips, high pitched voice, microcephaly	Het *SRCAP* variant, NM_006662.3: c.7303C>T p.Arg2435*
16	M	0.9	na	-5.6	-15.5	-10.6	-4.6	-2.7	-0.4	14.6	nd	nd	Primordial dwarfism, frequent infections, developmental delay	Hom *PCNT* variant c.5180dupA p.Asn1727LysfsTer14
17a	M	10.0	na	-4.5	-8.7	-10.9	-4.2	2.3	-0.5	0.1	nd	nd	Prominent nose, micrognathia, microdontia, hypo- and hyperpigmentation, cutis marmorata, café-au-lait spots, curved fingers, developmental delay	Hom *PCNT* variant NM_006031.6: c.3109G>T p.Glu1037*

17b	F	0.75	na	-5.5	-7.8	-8.3	-1.7	3.8	1.2	14	nd	nd	Primordial dwarfism	
18	M	10.9	na	-4.4	-6.3	-6.5	-3.3	-0.2	-0.7	1.2	24.5	nd	Primordial dwarfism	Hom *PCNT* variant NM_006031.6: c.721-2A>G p.?
19	F	4.3	2.5	500g (26 wks)	-8.5	-8.4	-4.7	-0.7	0.5	nd	nd	nd	Developmental delay, scoliosis, partial empty sella	Hom *PCNT* variant NM_006031.6: c.3608_3840del p.Pro1204Glyfs*11, Confirmed at RNA level
20	F	9.7	1.9	-2.4	-5.9	-4.5	-1.3	-5.2	-1.3	0.2, 1.1, 3.9	3.86	nd	Developmental delay, does not talk, convulsions, mental retardation, ataxia, walking with support	Hom *WDR4* variant NM_018669.6: c.428G>A p.Gly143Glu
21	M	11.8 14.9 15.7 18.0	2.7	Low	-3.7 -4.1 -3.4 -2.0	-3.9	-2.2	-0.6, -1.5	-0.5, <-2.0		21	nd	Developmental delay (IQ 78)	Het *GHSR* variant (VUS) NM_198407.2: c.1049C>G p.Thr350Ser
22	M	9.2	3.0	-4.4	-4.1	-5.4	-2.4	-1.9	-1.7	na	6.3	nd	Developmental delay, small pituitary	No causal variant

Abbreviations: BA, bone age; BW, birth weight; FSGS: focal segmental glomerulosclerosis. hc, head circumference; Het, heterozygous; Homozyg, homozygous; IGF gen test: IGF generation test; na, not available; nd, not done; resp, response

309 Supplementary Table S3. Subgroup 2b; Clinical and biochemical features and genetic findings in subjects with syndromic short stature

310

Patient number	Sex	Age (yrs)	BA delay (yrs)	BW SDS	Height SDS	HC SDS	BMI SDS	IGF-I SDS	IGFBP-3 SDS	GH basal (ng/mL)	GH max (ng/mL)	IGF gen test (ng/mL)	Clinical features	Genetic findings
23	F	2.9	1.6	-6.1	-5.2	-1.3	0.5	<-2.0	-4.3	3.5, 2.0	20	No response	Rhizomelia, frontal bossing, hair depigmented and sparse, atrial septum defect, patent ductus arteriosus	17p13.3 microdeletion (525-1,922,715)x1, (2,024,217-2,262,703)x1 Arr[hg19]
24	M	12.4	2.0	-1.7	-3.3	-2.8	-2.1	-0.7	-3.0	1.2	11.7	nd	Diarrhoea, eczema, dry discoloured hair	Hom *TTC37* variant c.4572G>A, p.Trp1524*
25a	M	13.0	0.9	-2.6	-3.6	-3.7	-2.6	-1.2	0.5	1.6	17	nd	Frequent infections, triangular face	Hom *SCUBE3* variant c.2599+2T>C p.?[a]
25b	F	8.5	0.7	-0.4	-3.9	-1.9	-1.4	-0.4	0.6	0.6	9.4	nd	Frequent infections, triangular face, idiopathic frequent ventricular extrasystoles	
26	F	8.5	0.7	-3.9	-3.1	-3.2	-2.3	-1.2	1.5	8.4	nd	nd	Triangular face, periorbital hyperpigmentation, short philtrum, thin elevated nasal bridge.	Het *NSD2* variant c.642dup, p.Asp215Argfs*10
27	M	8.8	3.0	-3.8	-3.3	-3.2	-0.5	1.4	1.3	0.3	11.2	nd	Intellectual delay, speech delay, bilateral hearing loss	Hom *RABGAP1* variant c.2789dup p.Asn930Lysfs*7
28	M	12.5	1.4	na	-4.1	-1.5	-1.1	-2.1	-3.6	na	22	+390 on high GH dose	none	No causal variant

29	F	17.7	0.6	-2.6	-5.3	-2.5	-3.7	-2.1	-0.7	0.5	17.2	nd	Frequent infections, developmental delay, absent toe, wide nasal bridge, retrognathia, hyperlordosis	No causal variant
30	F	13.5	3.0	-3.8	-7.0	-2.2	-3.8	-3.0	1.3	0.14	13.1	nd	Almond eyes, retrognathia, prominent nose, thin upper lip, absent pubertal signs	No causal variant
31	M	10.0	4.0	-2.8	-3.8	-2.5	-1.5	-0.9	1.1	0.4	7.0	nd	Bilateral hearing loss, developmental delay, triangular face, hyperlordosis, low-set ears	No causal variant

311 Abbreviations: BA, bone age; BW, birth weight; hc, head circumference; Het, heterozygous; Homozyg, homozygous; IGF gen test, IGF generation test; na, not available; nd, not done;

312 [a]c.2599+2T>C is predicted to result in multiple aberrantly processed transcripts, with p.Asn801Thrfs∗127 representing the prevalent out-of-frame product [25].

313

Supplementary Table 4. Subgroup 2c; Clinical, biochemical and genetic features of subjects with nonspecific growth hormone deficiency

Patient number	Sex	Age (yrs)	BA delay (yrs)	BW SDS	Height SDS	HC SDS	BMI SDS	IGF-I SDS	IGFBP-3 SDS	GH basal (ng/mL)	GH max (ng/mL)	IGF gen test (ng/mL)	Clinical features	Genetic findings
32	M	3.75	1.5 (at 4.8 yrs)	0.4	-4.7	-1.6	0.0	<-2	-2.6	1.0	6.8, 3.3	>15.5	Poor response to rhGH, possible partly to poor adherence.	No causal variant
33	M	7.3	3.5	-1.9	-4.5	-0.8	1.4	-1.5	-2.9	0.4	2.2, 0.6	nd	Good response to rhGH	No causal variant
34	M	2.8	0.8	-1.7	-3.8	-2.1	-0.1	<-1.7	-1.4	0.1	5.5, 0.23	Normal IGF-I and IGFBP-3 after 3 m rhGH	Eczema, curly hair, candidiasis of toenails. MRI: small adenohypophysis, small pituitary stalk, ectopic neurohypophysis	No causal variant

Abbreviations: BA, bone age; Nd, not done; m, months; rhGH, recombinant human growth hormone

References

1 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, 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. Genet Med 2015;17:405-424.

2 Biesecker LG, Harrison SM, ClinGen Sequence Variant Interpretation Working G: The ACMG/AMP reputable source criteria for the interpretation of sequence variants. Genet Med 2018;20:1687-1688.

3 Griffith E, Walker S, Martin CA, Vagnarelli P, Stiff T, Vernay B, et al.: Mutations in pericentrin cause Seckel syndrome with defective ATR-dependent DNA damage signaling. Nat Genet 2008;40:232-236.

4 Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J: A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 2014;46:310-315.

5 Elizondo LI, Cho KS, Zhang W, Yan J, Huang C, Huang Y, et al.: Schimke immuno-osseous dysplasia: SMARCAL1 loss-of-function and phenotypic correlation. J Med Genet 2009;46:49-59.

6 Ioan DM, Fryns JP: Floating-Harbor syndrome in two sisters: autosomal recessive inheritance or germinal mosaicism? Genet Couns 2003;14:431-433.

7 Karaer K, Karaoguz MY, Ergun MA, Yesilkaya E, Bideci A, Percin EF: Floating-Harbor syndrome: a first female Turkish patient? Genet Couns 2006;17:465-468.

8 Hood RL, Lines MA, Nikkel SM, Schwartzentruber J, Beaulieu C, Nowaczyk MJ, et al.: Mutations in SRCAP, encoding SNF2-related CREBBP activator protein, cause Floating-Harbor syndrome. Am J Hum Genet 2012;90:308-313.

9 Ko J, Pomerantz JH, Perry H, Shieh JT, Slavotinek AM, Oberoi S, et al.: Case Report of Floating-Harbor Syndrome With Bilateral Cleft Lip. Cleft Palate Craniofac J 2020;57:132-136.

10 Reschen M, Kini U, Hood RL, Boycott KM, Hurst J, O'Callaghan CA: Floating-Harbor syndrome and polycystic kidneys associated with SRCAP mutation. Am J Med Genet A 2012;158A:3196-3200.

11 Seifert W, Meinecke P, Kruger G, Rossier E, Heinritz W, Wusthof A, et al.: Expanded spectrum of exon 33 and 34 mutations in SRCAP and follow-up in patients with Floating-Harbor syndrome. BMC Med Genet 2014;15:127.

12 Yagi H, Takagi M, Narumi S, Hasegawa T, Nishimura G, Hasegawa Y: Stippled calcification in an infant with a recurrent SRCAP gene mutation. Am J Med Genet A 2016;170A:1088-1091.

13 Zhang S, Chen S, Qin H, Yuan H, Pi Y, Yang Y, et al.: Novel genotypes and phenotypes among Chinese patients with Floating-Harbor syndrome. Orphanet J Rare Dis 2019;14:144.

14 Shaheen R, Abdel-Salam GM, Guy MP, Alomar R, Abdel-Hamid MS, Afifi HH, et al.: Mutation in WDR4 impairs tRNA m(7)G46 methylation and causes a distinct form of microcephalic primordial dwarfism. Genome Biol 2015;16:210.

15 Trimouille A, Lasseaux E, Barat P, Deiller C, Drunat S, Rooryck C, et al.: Further delineation of the phenotype caused by biallelic variants in the WDR4 gene. Clin Genet 2018;93:374-377.

16 Chen X, Gao Y, Yang L, Wu B, Dong X, Liu B, et al.: Speech and language delay in a patient with WDR4 mutations. Eur J Med Genet 2018;61:468-472.

17 Braun DA, Shril S, Sinha A, Schneider R, Tan W, Ashraf S, et al.: Mutations in WDR4 as a new cause of Galloway-Mowat syndrome. Am J Med Genet A 2018;176:2460-2465.

18 Sari S, Sari E, Akgun V, Ozcan E, Ince S, Saldir M, et al.: Measures of pituitary gland and stalk: from neonate to adolescence. J Pediatr Endocrinol Metab 2014;27:1071-1076.

19 Pirozzi F, Lee B, Horsley N, Burkardt DD, Dobyns WB, Graham JM, Jr., et al.: Proximal variants in CCND2 associated with microcephaly, short stature, and developmental delay: A case series and review of inverse brain growth phenotypes. Am J Med Genet A 2021;185:2719-2738.

20 Baker EK, Brewer CJ, Ferreira L, Schapiro M, Tenney J, Wied HM, et al.: Further expansion and confirmation of phenotype in rare loss of YWHAE gene distinct from Miller-Dieker syndrome. Am J Med Genet A 2023;191:526-539.

21 Denomme-Pichon AS, Collins SC, Bruel AL, Mikhaleva A, Wagner C, Vancollie VE, et al.: YWHAE loss of function causes a rare neurodevelopmental disease with brain abnormalities in human and mouse. Genet Med 2023:100835.

22 Fabre A, Martinez-Vinson C, Roquelaure B, Missirian C, Andre N, Breton A, et al.: Novel mutations in TTC37 associated with tricho-hepato-enteric syndrome. Hum Mutat 2011;32:277-281.

23 Bourgeois P, Esteve C, Chaix C, Beroud C, Levy N, consortium Tc, et al.: Tricho-Hepato-Enteric Syndrome mutation update: Mutations spectrum of TTC37 and SKIV2L, clinical analysis and future prospects. Hum Mutat 2018;39:774-789.

24 Gao J, Hu X, Hu W, Sun X, Chen L: Novel TTC37 mutations in a patient with Trichohepatoenteric syndrome: a case report and literature review. Transl Pediatr 2022;11:1050-1057.

25 Lin YC, Niceta M, Muto V, Vona B, Pagnamenta AT, Maroofian R, et al.: SCUBE3 loss-of-function causes a recognizable recessive developmental disorder due to defective bone morphogenetic protein signaling. Am J Hum Genet 2021;108:115-133.

26 Oh RY, Deshwar AR, Marwaha A, Sabha N, Tropak M, Hou H, et al.: Biallelic loss-of-function variants in RABGAP1 cause a novel neurodevelopmental syndrome. Genet Med 2022;24:2399-2407.

27 Kurata H, Terashima H, Nakashima M, Okazaki T, Matsumura W, Ohno K, et al.: Characterization of SPATA5-related encephalopathy in early childhood. Clin Genet 2016;90:437-444.
28 Buchert R, Nesbitt AI, Tawamie H, Krantz ID, Medne L, Helbig I, et al.: SPATA5 mutations cause a distinct autosomal recessive phenotype of intellectual disability, hypotonia and hearing loss. Orphanet J Rare Dis 2016;11:130.
29 Lin Z, Zhao J, Nitoiu D, Scott CA, Plagnol V, Smith FJ, et al.: Loss-of-function mutations in CAST cause peeling skin, leukonychia, acral punctate keratoses, cheilitis, and knuckle pads. Am J Hum Genet 2015;96:440-447.
30 Isik E, Haliloglu B, van Doorn J, Demirbilek H, Scheltinga SA, Losekoot M, et al.: Clinical and biochemical characteristics and bone mineral density of homozygous, compound heterozygous and heterozygous carriers of three novel IGFALS mutations. Eur J Endocrinol 2017;176:657-667.