Clinical and Molecular Description of 16 Families With Heterozygous *IHH* Variants

Lucía Sentchordi-Montané,[1,2,3,4] Sara Benito-Sanz,[1,5] Miriam Aza-Carmona,[1,4,5] Arrate Pereda,[6] Manuel Parrón-Pajares,[4,7] Carolina de la Torre,[1] Gabriela A. Vasques,[8,9] Mariana F.A. Funari,[9] André M. Travessa,[10] Patrícia Dias,[10] Larisa Suarez-Ortega,[11] Jesús González-Buitrago,[12] Nancy Elizabeth Portillo-Najera,[13] Isabel Llano-Rivas,[14] María Martín-Frías,[15] Joaquín Ramírez-Fernández,[16] Jaime Sánchez del Pozo,[17] Lucía Garzón-Lorenzo,[17] Gabriel A. Martos-Moreno,[18,19,20] Cristina Alfaro-Iznaola,[21] Inés Mulero-Collantes[22] Pablo Ruiz-Ocaña,[23] Paula Casano-Sancho,[24] Ana Portela,[25] Lorea Ruiz-Pérez,[26] Angela del Pozo,[3,5] Elena Vallespín,[3,5] Mario Solís,[3] Antônio M. Lerario,[8,27] Isabel González-Casado,[4,28] Purificación Ros-Pérez,[29] Guiomar Pérez de Nanclares,[6] Alexander A. L. Jorge,[8,9] and Karen E. Heath[1,4,5]

[1]Institute of Medical and Molecular Genetics (INGEMM); IdiPAZ, Hospital Universitario La Paz, 28046 Madrid, Spain; [2]Department of Pediatrics, Hospital Universitario Infanta Leonor, Madrid 28031, Spain; [3]Department of Pediatrics, School of Medicine, Complutense University of Madrid, 28040, Madrid, Spain; [4]Skeletal Dysplasia Multidisciplinary Unit (UMDE), Hospital Universitario La Paz, 28046, Madrid, Spain; [5]CIBERER, ISCIII, 28029 Madrid, Spain; [6]Rare Diseases Research Group, Molecular (Epi)Genetics Laboratory, BioAraba Health Research Institute, Hospital Universitario Araba-Txagorritxu, 01009 Vitoria-Gasteiz, Araba, Spain; [7]Department of Radiology, Hospital Universitario La Paz, 28046 Madrid, Spain; [8]Unidades de Endocrinologia Genetica (LIM/25), Hospital das Clinicas da Faculdades de Medicina, Universidades de São Paulo, São Paulo 01246-903, Universidades de São Paulo, São Paulo 01246-903, Brazil; [9]Unidade de Endocrinologia do Desenvolvimento, Laboratorio de Hormonios e Genetica Molecular (LIM42), Hospital das Clinicas da Faculdade de Medicina, Universidade de São Paulo (USP), São Paulo 05403-000, Brazil; [10]Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, 1649-028, Lisbon, Portugal; [11]Division of Endocrinology, Hospital Sant Joan de Déu, 08950 Esplugnes de Llobregat, Barcelona, Spain; [12]Department of Pediatrics. Hospital San Pedro de Alcántara, 10003 Cáceres, Spain; [13]Department of Pediatrics, Hospital Alto Deba, 20500 Arrasate, Guipuzkoa, Spain; [14]Osakidetza Basque Health Service, Cruces University Hospital Department of Genetics, 48903 Barakaldo, Bizkaia, Spain; [15]Department of Pediatric Endocrinology, Hospital Universitario Ramón y Cajal, 28034 Madrid, Spain; [16]Department of Pediatrics, Hospital Universitario Príncipe de Asturias, 28805 Alcalá de Henares, Madrid, Spain; [17]Department of Pediatric Endocrinology, Hospital Universitario Doce de Octubre, 28041, Madrid, Spain; [18]Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación Sanitaria La Princesa, 28009 Madrid, Spain; [19]Department of Pediatrics, School of Medicine, Universidad Autónoma de Madrid, 28029 Madrid, Spain; [20]CIBEROBN, ISCIII, 28029 Madrid, Spain; [21]Department of Pediatrics, Hospital Universitario Rey Juan Carlos, 28933 Móstoles, Madrid, Spain; [22]Department of Pediatrics, Hospital Universitario Río Hortega, 47012 Valladolid, Spain; [23]Department of Pediatrics, Hospital Universitario Puerta del Mar, 11009 Cádiz, Spain; [24]Department of Pediatric Endocrinology, Institut de Recerca Pediàtrica, Hospital Sant Joan de Déu, University of Barcelona, 08950 Espluges de Llobregat, Barcelona, Spain and CIBERDEM, ISCIII, 28029 Madrid, Spain; [25]Department of Pediatric Endocrinology, Pediatric Unit, Hospital Universitario Nuestra Señora de Candelaria, 38010 Santa Cruz de Tenerif, Spain; [26]Department of Pediatric Endocrinology, Hospital General Universitario de Alicante, 03010, Alicante, Spain; [27]Department of Internal Medicine, Division of Metabolism, Endocrinology and Diabetes, University of Michigan, Ann Arbor, MI 48109, US; [28]Department of Pediatric Endocrinology, Hospital Universitario La Paz, 28046, Madrid, Spain; and [29]Department of Pediatrics, Hospital Universitario Puerta de Hierro Majadahonda, 28222 Majadahonda, Madrid, Spain

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 27 January 2020. Accepted 17 April 2020.
First Published Online 20 April 2020.
Corrected and Typeset 27 June 2020.

Abbreviations: ACFD, acrocapitofemoral dysplasia; BDA1, brachydactyly type A1; ISS, idiopathic short stature; *IHH, Indian hedgehog gene*; NGS, next-generation sequencing; Ptc, Patched; VUS, variant of unknown significance.

ORCiD numbers: 0000-0002-4083-3352 (L. Sentchordi-Montané); 0000-0002-6455-8682 (G. A. Vasques); 0000-0003-4316-4999 (M. F.A. Funari); 0000-0002-8336-6432 (A. M. Lerario); 0000-0003-2567-7360 (A. A. L. Jorge); 0000-0002-5816-7044 (K. E. Heath).

Context: Heterozygous variants in the Indian hedgehog gene (*IHH*) have been reported to cause brachydactyly type A1 and mild hand and feet skeletal anomalies with short stature. Genetic screening in individuals with short stature and mild skeletal anomalies has been increasing over recent years, allowing us to broaden the clinical spectrum of skeletal dysplasias.

Objective: The objective of this article is to describe the genotype and phenotype of 16 probands with heterozygous variants in *IHH*.

Patients and Methods: Targeted next-generation sequencing or Sanger sequencing was performed in patients with short stature and/or brachydactyly for which the genetic cause was unknown.

Results: Fifteen different heterozygous *IHH* variants were detected, one of which is the first reported complete deletion of *IHH*. None of the patients showed the classical phenotype of brachydactyly type A1. The most frequently observed clinical characteristics were mild to moderate short stature as well as shortening of the middle phalanx on the fifth finger. The identified *IHH* variants were demonstrated to cosegregate with the short stature and/or brachydactyly in the 13 probands whose family members were available. However, clinical heterogeneity was observed: Two short-statured probands showed no hand radiological anomalies, whereas another 5 were of normal height but had brachydactyly.

Conclusions: Short stature and/or mild skeletal hand defects can be caused by *IHH* variants. Defects in this gene should be considered in individuals with these findings, especially when there is an autosomal dominant pattern of inheritance. Although no genotype-phenotype correlation was observed, cosegregation studies should be performed and where possible functional characterization before concluding that a variant is causative. **(*J Clin Endocrinol Metab* 105: 1–13, 2020)**

Freeform/Key Words: *IHH*, brachydactyly, short stature, NGS

Chondrogenesis is the fundamental biological process that drives linear growth and therefore stature in children, with heritable factors playing a paramount role in this process. Short stature is one of the most common referrals in pediatric endocrinology clinics. After excluding common causes, many are labeled as having idiopathic short stature (ISS), a clinical heterogeneous entity. During the last decade, clinicians and researchers have begun to unravel the genetic causes underlying ISS. On detailed clinical and radiological examination, some of these individuals actually have mild body disproportion and minor skeletal defects (1). Pathogenic variants in genes involved in various signaling pathways have been detected by us and others, in specific subgroups of individuals who share particular clinical features, short stature with or without limb shortening, and mild skeletal anomalies including brachydactyly, such as heterozygous variants in the aggrecan gene (*ACAN*), the natriuretic peptide receptor 2 gene (*NPR2)* encoding natriuretic peptide receptor B (NPR-B) and its ligand C-natriuretic peptide (CNP) encoded by the natriuretic peptide precursor C gene (*NPPC)*, and Indian hedgehog gene (*IHH)* (2-8). The detailed clinical and radiological examination and the implementation of next-generation sequencing (NGS) in the genetic studies of pediatric short stature cases has led over recent years to our increasing detection of variants in *IHH*.

IHH is a peptide that is transported to the endoplasmic reticulum and Golgi apparatus, where it undergoes autoprocessing. There it undergoes cleavage into 2 fragments, the N-terminal, the functional signaling molecule, and the C-terminal, which regulates autoprocessing. After processing of the N-terminal part by cholesterol and palmitate, IHH is secreted from the producing cells and binds to the membrane receptor protein Patched (Ptc). The membrane protein, smoothened, then receives the signal, and transfers it into the nucleus by the transcription factor Gli (Sasai [9], review), where it binds to its target genes. IHH is predominantly expressed in the developing skeleton. It is expressed in the prehypertrophic chondrocytes of

cartilage and coordinates proliferation and differentiation of chondrocytes during endochondral ossification (10) and is directly required for the osteoblast lineage in the developing long bones along with other factors such as bone morphogenetic proteins to induce osteoblast differentiation (11).

Brachydactyly type A1 (MIM 112500; BDA1), characterized by shortened or absent middle phalanges in digits along with short stature, is associated with heterozygous variants in *IHH* (12). Since the first description, many families have been reported with variable expressivity and penetrance (13-17). Homozygous variants cause another dysplasia, acrocapitofemoral dysplasia (ACFD, MIM 607778), characterized by cone-shaèd epiphyses in phalanges, proximal femurs and tibias (18). Initially, variants causing BDA1 were reported to occur only in the N-terminal active fragment of *IHH* (19), whereas variants causing ACFD were located in the distal N- and C-terminal regions (18). However, during recent years we have detected heterozygous variants throughout the gene in families not with typical BDA1 but with short stature and brachydactyly and, once again, variable expressivity (8). To date no other disorders have been associated with variants in *IHH* (9).

Here, we describe the broad spectrum of the clinical and radiological characteristics of 16 probands and their family members with heterozygous *IHH* variants. We also report the identification of the first complete deletion of *IHH* in a patient with short stature and brachydactyly.

Cohorts and Methods

All participants provided informed consent for the performed studies and ethical approval was obtained from local ethical committees. Probands were referred by pediatric endocrinologists or clinical geneticists for: group 1: proportionate/disproportionate short stature and mild skeletal defects and/or a parent with disproportionate short stature; group 2: brachydactyly of unknown cause; or, group 3: ISS. All had a complete physical exam and a skeletal survey. Endocrine disorders including somatotropic axis–related conditions were excluded by biochemical analysis. All participants had also been previously excluded for *SHOX* defects using Multiplex Ligation–dependent Probe Amplification (MLPA) (P018G2, MRC Holland) and DNA sequencing.

Blood samples were extracted from the proband and family members, when available. The probands were analyzed using a custom designed skeletal dysplasia NGS panel, SKELETALSEQ.V4-8 (n = 327-416 genes), a custom-designed short-stature NGS panel (8) or in 3 cases (probands 1, 2, and 4) by direct Sanger sequencing. Genes included in these panels are available on request. The identified variants were assessed for amino acid conservation using in silico pathogenicity prediction analysis: CADD V1.4 (http://cadd.gs.washington.edu/), SIFT, Polyphen, MutationTaster, various splicing programs available in Alamut V2.14 (Interactive Biosoftware); and allelic frequencies in gnomAD (https://gnomad.broadinstitute.org/). Copy number variant (deletion/duplication) analysis was performed by in-house software (unpublished). All variants detected by NGS were subsequently validated by Sanger sequencing, as was family testing. Kinship was confirmed using microsatellite marker analysis (Devyser Complete quantitative fluorescence–polymerase chain reaction). The *IHH* deletion was confirmed by a single-nucleotide polymorphism array (Infinium CytoSNP-850K v1.2 BeadChip, Illumina) but because of low probe density in this region we further characterized the deletion using a custom-designed *IHH* MLPA assay (probe sequences are available on request).

After the identification of an *IHH* variant, personal and familial records, anthropometric measures as well as the evaluation of the skeletal survey were reviewed by the same clinician (L.S-M.) and radiologist (M.P-P.).

Results

Fifteen different *IHH* variants were detected in 16 probands, 12 not been previously described (Table 1), whereas the variants present in probands 2, 4, and 15 have been previously described (8, 14). Using the American College of Medical Genetics and Genomics guidelines for variant classification (20), 2 of the variants have been classified as pathogenic, 5 as likely pathogenic, and the remaining 8 as variants of unknown significance (VUS). The variants are localized throughout the protein (both N- and C- terminal domains). The pedigrees and genotypes of the 16 families are shown in Fig. 1. Cosegregation of genotype and phenotype was demonstrated in 13 families, whereas family testing was unavailable for the remaining 3 families (probands 9, 14, and 15). No other pathogenic or likely pathogenic variant was detected in the NGS panels, nor any strong VUS candidate.

Interestingly, a complete deletion of *IHH* was identified in proband 16, initially by copy number variant analysis of the NGS skeletal dysplasia panel and subsequently confirmed by a single-nucleotide polymorphism array and a self-designed *IHH* MLPA (Fig. 2). The

Table 1. Molecular details of *IHH* variants identified in the cohort

Proband	Mutation	Mutation class	Exon	gnomAD, all, %	CADD V1.4/ SIFT/Polyphen/ MutationTaster	Protein domain	ACMG
1	c.228_229delinsAA p.(Arg77Ser)	Missense	1	Absent	23.1/D/PD/NA	N-terminal domain	VUS (PM2, PP3, PP4)
2	c.283_285del p.(Glu95del)[a]	Inframe deletion	1	Absent	NA	N-terminal domain	Likely pathogenic (PM2, PM4, PP1, PP4, PP5)
3	c.391G>C p.(Glu131Gln)	Missense	2	Absent	32/D/PD/DC	N-terminal domain	Likely pathogenic (PM2, PM5, PP2, PP3, PP4)
4	c.446G>A p.(Arg149His)[b]	Missense	2	Absent	35/D/PD/DC	N-terminal domain	VUS (PM2, PP2, PP3, PP4, PP5)
5	c.470A>G; p.(Asp157Gly)	Missense	2	Absent	33/D/PD/DC	N-terminal domain	VUS (PM2, PP2, PP3, PP4)
6	c.482_510del p.(Asn161Serfs*6)	Frameshift	2	Absent	NA	N-terminal domain	Likely pathogenic (PVS1, PM2)
7	c.531G>A p.(Trp177*)	Nonsense	2	Absent	41/NA/NA/NA	N-terminal domain	Likely pathogenic (PVS1, PM2)
8	c.541del p.(Glu181Serfs*43)	Frameshift	2	Absent	NA	N-terminal domain	Pathogenic (PVS1, PM2, PP4)
9	c.568_570del p.(Val190del)	Inframe deletion	2	Absent	NA	N-terminal domain	VUS (PM2, PM4)
10	c.685G>A p.(Val229Met)	Missense	3	Absent	27/D/PD/DC	C-Hint domain	VUS (PM2, PP2, PP3)
11	c.823C>A p.(His275Asn)	Missense	3	Absent	25.8/D/PD/DC	C-Hint domain	VUS (PM2, PP1, PP2, PP3)
12	c.887_890del p.(Ser296Thrfs*68)	Frameshift	3	Absent	NA	C-Hint domain	Likely pathogenic (PVS1, PM2)
13, 14	c.892G>A p.(Val298Met)	Missense	3	0.002601	33/D/PD/DC	C-Hint domain	VUS (PP1, PP2, PP3, PP4)
15	c.949G>A p.(Val317Met)[b]	Missense	3	0.000416	26.3/D/PD/DC	C-Hint domain	VUS (PP2, PP3)
16	Complete deletion	Deletion	–	Absent	NA	N/A	Pathogenic (PVS1, PS2, PM2)

Coordinates are based on NM_002181.4 transcript.
Abbreviations: ACMG, American College of Medical Genetics and Genomics; all, allelic frequency; DC, disease causing; del, deleterious; NA, not analyzed by this/these methods; N/A: not applicable; PD, probably damaging; VUS: variant of unknown significance.
In silico analysis: CADD V1.4: value greater than 15 is considered to be deleterious.
[a]Described previously (14).
[b]These variants have been previously described (8).

deletion, hg19:2:219,923,348-219,942,928x1, was shown to include only *IHH*. The deletion was also detected in the similarly affected father.

The clinical characteristics and hand radiographs of the 16 probands (5 male, 11 female) are shown in Table 2 and Fig. 3, respectively. The cohort consisted of 12 Spanish patients, 3 Brazilian, and 1 Portuguese. Ages at the time of study ranged from 1.5 to 17 years (median, 12.8 years) and the average height SD was –2 (range, 0.37 to –3.8 SD). Average height SD of the affected parents was –2.54 (range, –0.8 to –4.7). Three probands were born small for gestational age for body length (23%). Unfortunately, complete anthropometric measurements were not available for all participants. Despite only one proband have a sitting height/height ratio SD greater than 2, the majority (10/12, 83%) presented with a sitting height/height ratio greater than 1 SD (21, 22). In contrast arm span/height ratio was less than 0.96 in 7 of 11 (63%). The principal characteristics observed in the 16 probands were short stature (68%) and shortening of the middle phalanx (75%) (Table 2, Fig. 3).

Discussion

The present study reports the clinical and molecular study of 16 probands with heterozygous mutations in *IHH*, including the identification of the first complete deletion of *IHH*. The deletion was identified in a girl (proband 16) with short stature (–3.2 SD), mild micromelia of the lower limbs, and shortening of the middle phalanges of the second and fifth fingers. The deletion is also present in her father, who shows a similar phenotype. Despite the complete absence of the gene, their phenotype is similar to that observed in the probands with nonsense or missense variants.

Although no functional characterization was performed, some of the variants identified in this cohort

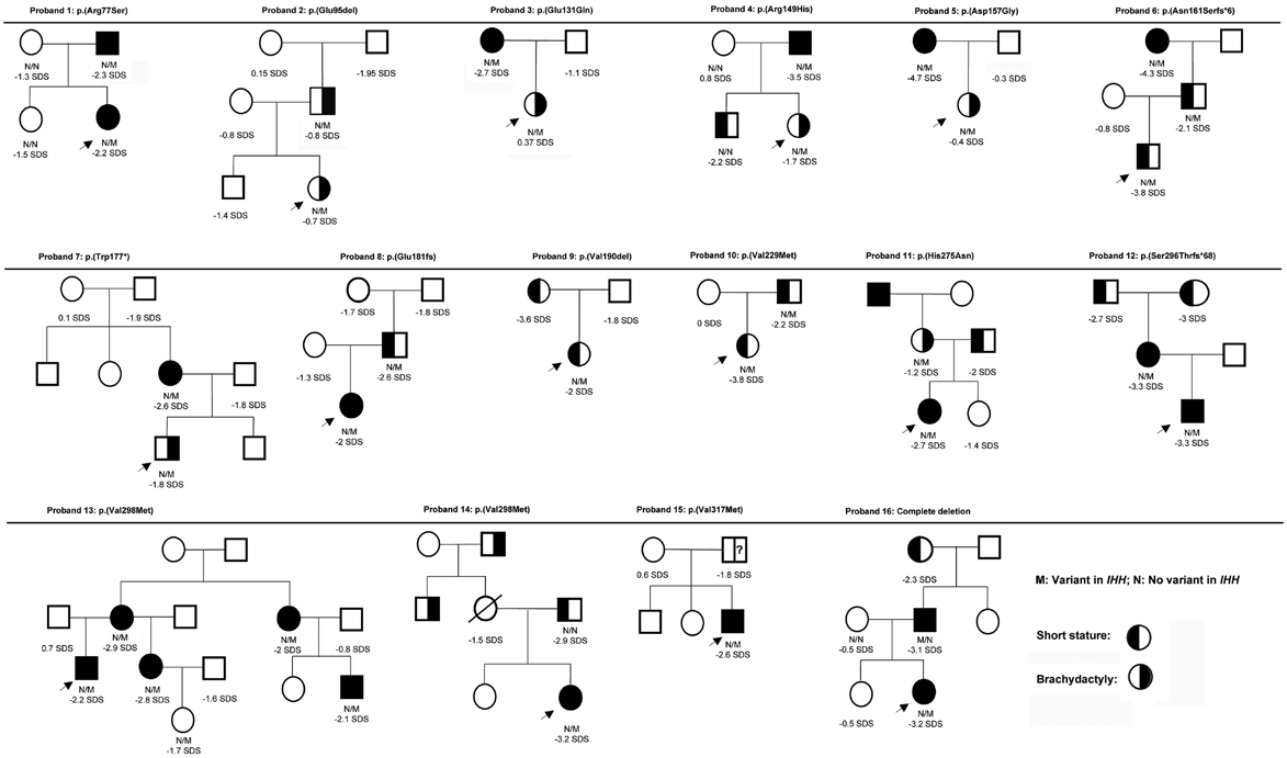

Figure 1. Pedigrees obtained from the 16 families affected carrying heterozygous mutations in the Indian hedgehog gene (*IHH*). The arrows indicate the probands. N indicates no *IHH* variant, M indicates *IHH* variant. Height SD is indicated where available. Short stature is indicated by left half-filled symbols, brachydactyly is indicated by right half-filled symbols. When phenotype is unknown a question mark has been placed inside the sex symbol.

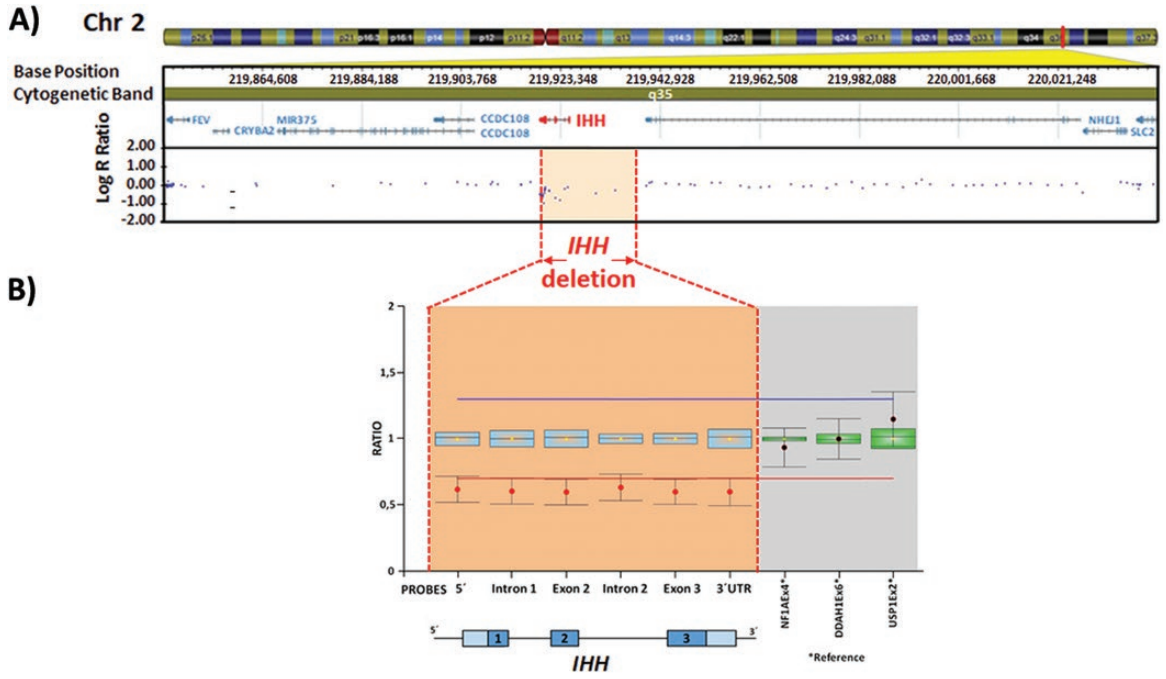

Figure 2. Characterization of the Indian hedgehog gene (*IHH*) deletion detected in proband 16. A, Single-nucleotide polymorphism (SNP) array (Infinium CytoSNP-850K v1.2 BeadChip, Illumina) showing the deletion hg19:2:219,923,348-219,942,928x1. Sequence coordinates are taken from GRCh37/hg19. B, Coffalyser plot of the custom-designed *IHH* multiplex ligation probe amplification, confirming the complete deletion (represented in the orange shaded box). Normal peaks were classified as having a ratio of 0.7 to 1.3 and deletions were classified as less than 0.7. Asterisks represent control probes, located in other chromosomes.

have convincing evidence for the pathogenicity. First, proband 3 and her mother have a missense variant in codon 131, c.391G>C; p.(Glu131Gln). Another variant at the same amino acid, c.391G>A; p.Glu131Lys, has been reported several times (12, 15, 23). This variant is one of the few that has been functionally characterized,

Table 2. Clinical, anthropometric, and radiological characteristics of the cohort

Proband	Variant	Sex	Geographic origin	Reason for genetic investigation	Age, y	Height SDS	Midparental target height SD	SGA (SD weight/ SD length)	Sitting height/ height (SD)[a]	Arm span/ height	Skeletal maturation (BA:CA)	Hand x-ray findings (shortening)	Feet x-ray findings (shortening)	Affected family members (height SD, brachydactyly ±)
1	c.228_229delinsAA p.(Arg77Ser)	F	Spain	2	13	–2.2	–1.6	No	NA	NA	+2	Left middle phalanx (second, fifth). Right middle phalanx (fifth). Left metacarpals (third, fourth). Right metacarpal (fourth, severe)	Bilateral metatarsal (third, fourth)	Father –2.3 Brachy +
2	c.283_285del p.(Glu95del)	F	Spain	2	11	–0.7	–0.8	Yes (Un/–2)	NA	NA	+2	Middle phalanx (all, more second and fifth). Distal phalanx fourth. Metacarpal fourth, fifth. Mild proximal phalanx first	No abnormal findings	Father –0.8 Brachy +
3	c.391G>C p.(Glu131Gln)	F	Spain	2	11.5	0.37	–2	No	1.59	0.95	+3	Middle phalanx (fifth, second, mild fourth). Distal phalanx (third). Bilateral metacarpal (fifth and third)	Fourth metatarsal	Mother –2.7 Brachy +
4	c.446G>A p.(Arg149His)	F	Spain	2	11	–1.7	–1.4	No	2.6	0.956	=	Middle phalanx (second, fifth). Distal phalanx (first, third)	Bilateral metatarsal (fourth). Left metatarsal (third). General phalanx shortening	Father –3.5 Brachy +

Table 2. Continued

Proband	Variant	Sex	Geographic origin	Reason for genetic investigation	Age, y	Height SDS	Midparental target height SD	SGA (SD weight/ SD length)	Sitting height/ height (SD)[a]	Arm span/ height	Skeletal maturation (BA:CA)	Hand x-ray findings (shortening)	Feet x-ray findings (shortening)	Affected family members (height SD, brachydactyly ±)
5	c.470A>G p(Asp157Gly)	F	Spain	1	9.5	–0.43	–2.16	No	1.4	0.94	+1.5	Middle phalanx (second, fifth). Distal phalanx (mild first, second, third, fourth). Fifth metacarpal short and wide	Bilateral metatarsal (fourth)	Mother –4.7 Brachy+
6	c.482_510del p.(Asn161Serfs*6)	M	Portugal	1	1.5	–3.8	–1.2	Yes (–0.49/–2.39)	NA	NA	=	Clinodactyly (fifth)	No abnormal findings	Father –2.1 Brachy–
7	c.531G>A p.(Trp177*)	M	Spain	1	11.3	–1.8	–2.1	No	1.2	0.93	=	Middle phalanx (second, fifth). Distal phalanx (first, third with premature physeal closure in third). Cone shaped epiphyses (third, fourth, fifth)	No abnormal findings	Mother –2.6 Brachy+
8	c.541del p.(Glu181Serfs*43)	F	Brazil	1	2.4	–2	–2.1	No	1.19	0.87	=	Short hands. Broad and short first metacarpal. Broad fifth metacarpal	Cone-shaped epiphyses in proximal phalanges of toes (second, third, fourth, fifth)	Father –2.6 Brachy–
9	c.568_570delGTC p.(Val190del)	F	Spain	1	11.5	–2.02	–2.8	No	1.14	1	+3	Middle phalanx (fifth). Distal phalanx (first, second, third, fifth)	No abnormal findings	NA

doi:10.1210/clinem/dgaa218

Table 2. Continued

Proband	Variant	Sex	Geographic origin	Reason for genetic investigation	Age, y	Height SDS	Midparental target height SD	SGA (SD weight/ SD length)	Sitting height/ height (SD)[a]	Arm span/ height	Skeletal maturation (BA:CA)	Hand x-ray findings (shortening)	Feet x-ray findings (shortening)	Affected family members (height SD, brachydactyly ±)
10	c.685G>A p.(Val229Met)	F	Brazil	3	12.9	–3.8	–1.3	No	1.1	NA	–1.5	No abnormal findings	No abnormal findings	Father –2.2 Brachy NA
11	c.823C>A p.(His275Asn)	F	Spain	1	7.3	–2.7	–1.6	No	1	0.97	–1.5	Middle phalanx (fifth, mild)	No abnormal findings	Mother –1.2 Brachy– Grandfather –4 Brachy–
12	c.887_890del p.(Ser296Thrfs*68)	M	Spain	1	5.5	–3.3	–2.4	Yes (–1.26/–2.66)	NA	0.89	–2	Short hands. No abnormal radiological findings	No abnormal findings	Mother –3.3 Brachy+
13	c.892G>A p.(Val298Met)	M	Spain	1	17	–2.1	–1.4	No	1.7	0.93	=	Middle phalanx (fifth mild)	No abnormal findings	Mother –2, Brachy– Sister –2.8, Brachy+ Nephew –1.7, Brachy– Mat. aunt –2.9, Brachy NA Cousin –2.2, Brachy–
14	c.892G>A p.(Val298Met)	F	Spain	1	12	–3.2	–2.29	No	–0.9	0.98	–2	Middle phalanx (fifth mild). Irregular metaphyses of middle and proximal phalanges (second, third, fourth, fifth). Cone-shaped epiphyses of middle phalanges (second and fifth)	Bilateral metatarsal (fourth and fifth)	NA
15	c.949G>A p.(Val317Met)	M	Brazil	3	7.5	–2.6	–0.6	No	0.35	NA	–2.5	Middle phalanx (fifth and mild second). Clinodactyly (fifth)	No abnormal findings	NA

Table 2. Continued

Proband	Variant	Sex	Geographic origin	Reason for genetic investigation	Age, y	Height SDS	Midparental target height SD	SGA (SD weight/ SD length)	Sitting height/ height (SD)[a]	Arm span/ height	Skeletal maturation (BA:CA)	Hand x-ray findings (shortening)	Feet x-ray findings (shortening)	Affected family members (height SD, brachydactyly ±)
16	Complete deletion	F	Spain	1	5.7	–3.2	–1.8	No	1.21	0.99	=	Middle phalanx (second, fifth)	No abnormal findings	Father –3.1 Brachy+
	Summary	11 F 5 M	12 Spain 3 Brazil 1 Portugal	Group 1:10 Group 2:4 Group 3:2	Medium age: 12.8 y	Medium height: –2 5 probands with normal height	Medium height: –2	3 Yes/13 No	1/12 Disproportion (>2 SD) 10/12 SD ≥ 1	7/11 Disproportion	5 advanced 6 equal 5 delayed	12 shortened middle phalanges 6 shortened distal phalanges 1 shortened proximal phalanges 5 shortened metacarpals 2 clinodactyly 2 cone-shaped epiphyses 2 no abnormal findings	10/16 No abnormal findings	AD: 13 NA: 3

Reasons for genetic investigation: group 1) Proportionate/disproportionate short stature and mild skeletal defects and/or a parent with disproportionate short stature; group 2) Brachydactyly of unknown cause; group 3) Idiopathic short stature. Skeletal maturation (BA, CA): + applies to advanced BA to CA with BA at least 1 year greater than CA; = means BA less than 1 year greater than or less than CA, – means BA delayed by more than 1 year less than CA. An arm span/height ratio of less than 0.965 was used to determine disproportionality. Hand/feet x-ray findings: shortening of the phalanges is indicated. Affected family members (height expressed in SD, brachy ±: brachydactyly present/not present).

Abbreviations: AD, autosomal dominant inheritance; BA, bone age; CA, chronological age; F, female; NA, not available. M, male; Mat, maternal; SGA, small for gestational age.

[a]Sitting height/height ratio is expressed following references of Fredriks et al (21).

doi:10.1210/clinem/dgaa218

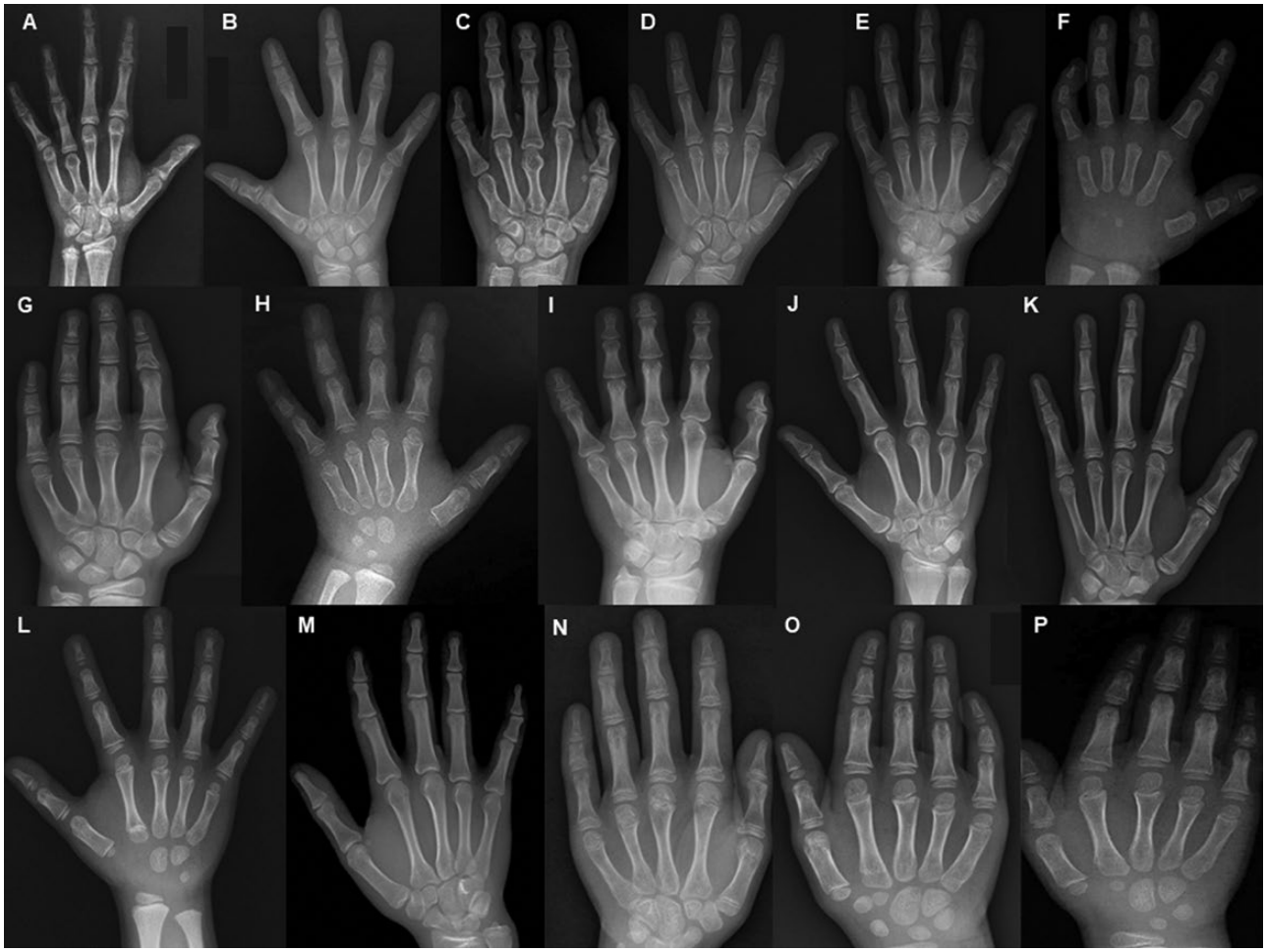

Figure 3. Hand radiographs from patients of the cohort (phalanx shortening and other special features). A, Patient 1: middle phalanx shortening (second and fifth) and severe fourth metacarpal shortening; B, Patient 2: Middle phalanx shortening (all, more second and fifth), distal phalanx shortening (fourth), mild fourth metacarpal shortening, mild first proximal phalanx shortening; C, Patient 3: Middle phalanx shortening (second, fifth, and mild fourth), fifth and third metacarpal shortening, distal phalanx shortening (third); D, Patient 4: Middle phalanx shortening (second and fifth) and distal phalanx shortening (first and third); E, Patient 5: Middle phalanx shortening (second and fifth), distal phalanx shortening (mild first, second, third, and fourth), fifth metacarpal short and wide; F, Patient 6: Clinodactyly (fifth); G, Patient 7: Middle phalanx shortening (second and fifth), distal phalanx shortening (first, third with premature physeal closure in third) cone-shaped epiphyses (third, fourth, and fifth); H, Patient 8: Short hands, broad and short first metacarpal, broad fifth metacarpal; I, Patient 9: Middle phalanx shortening (fifth), distal phalanx shortening (first, second, third, and fifth); J, Patient 10: No abnormal findings; K, Patient 11: Middle phalanx shortening (fifth); L, Patient 12: Short hands, no abnormal radiological findings; M, Patient 13: Mild middle phalanx shortening (fifth); N, Patient 14: Middle phalanx shortening (fifth mild), irregular metaphyses of middle and proximal phalanges (second, third, fourth, and fifth) with cone-shaped epiphyses of middle phalanges (second and fifth); O, Patient 15: Middle phalanx shortening (fifth and mild second) and clinodactyly (fifth); and P, Patient 16: Middle phalanx shortening (second and fifth).

demonstrating that this variant affects Hedgehog (Hh) binding to the receptor Ptc1, reducing its capacity to induce cellular differentiation, and thus, confirming its pathogenicity (23). The p.(Glu131Gln) variant identified in proband 3 may also impair Hh binding to Ptc.

Second, proband 2 has an inframe deletion variant, p.(Glu95del), which has been previously described (14). Residue Glu95 is estimated to be located on the edge of a groove important for the interaction between *IHH* and its receptor Ptc. The Glu95 deletion predicted the loss of a loop on the edge of this groove, suggesting that the deletion of this conserved amino acid is the cause of BDA1 in the family described by them. Two different variants affecting amino acid 95, p.(Glu95Lys) and p.(Glu95Gly), had been previously reported in individuals with BDA1 (10, 24). The family with the p.(Gly95Lys) variant had a more severe phenotype, whereas no clinical details were reported for the other case. We still do not know completely how *IHH* interacts with Ptc, and it is difficult to predict why different variants have a different effect on digit formation. But it does appear that this highly conserved Glu95 residue is important for correct *IHH* signaling.

Third, proband 1, with an indel resulting in a missense variant, c.228_229delinsAA; p.(Arg77Ser), has short stature and an uncommon finger phenotype, asymmetrical shortening of the middle phalanges and metacarpals and shortening of the third and fourth metatarsal.

Interestingly, the same amino acid substitution but different nucleotide change, c.229C>A; p.(Arg77Ser) (rs142036701), was the unique *IHH* variant described in a genome-wide association study of human growth in which 200 000 coding variants were studied in 711 428 individuals (25). This variant has a minor allele frequency of 0.08%. They showed that this variant and another 82 height-associated variants with minor allele frequencies in the range of 0.1% to 4.8% had effects of up to 2 cm per allele, 10 times greater than the average effect of common variants.

Last, proband 9 carries an inframe deletion, c.568_570del; p.(Val190del), classified as a VUS. A homozygous variant affecting the same codon, c.569T>C; p.(Val190Ala), was reported in 3 members of a consanguineous family with ACFD (18). Two heterozygous carriers of the mutation in the same family showed relative shortening of the metacarpals and proximal phalanges. Although no functional characterization has been performed for this variant, amino acid 190 may be a critical region in *IHH*.

The classical phenotype caused by heterozygous variants in *IHH,* BDA1, characterized by short stature and marked shortening of the middle phalanges, which are frequently rudimentary or fused to the terminal phalanges, was not present in any of the 16 probands. In this cohort, the observed phenotype was much milder. Short stature was present in 68% of the probands and 75% had shortening of the middle phalanx of the fifth finger. Nevertheless, both traits were present together in only 7 probands (43%). Upper limb shortening was observed in 7 of 11 (63%) probands, whereas lower limb micromelia was strictly observed in only 1 of 12. However, 10 of 12 of the probands presented with a sitting height/height ratio greater than 1 SD. Thus, our data suggest that mild micromelia might be observed and that upper limbs may be more affected than lower limbs. Other hand anomalies were also observed, including distal phalanx shortening, metacarpal shortening, isolated clinodactyly, and cone-shaped epiphysis. In total, 2 probands had short stature without the characteristic phalanx shortening, 9 had short stature with variable finger anomalies, and 5 had normal stature with phalanx shortening. Thus, clinical heterogeneity is associated with heterozygous *IHH* variants.

Intrafamilial variability and incomplete penetrance was also observed but may be explained, in part, by the age of the probands. Five of the probands (patients 2, 3, 4, 5, and 7) presented with typical features of brachydactyly but normal stature. Proband 7 and his mother have a nonsense variant in *IHH*. The proband has only brachydactyly whereas his mother has short stature and brachydactyly. Incomplete penetrance may be occurring or simply the child is young and has not reached adult height yet. Proband 2 and his father carry a missense mutation; both presented with isolated brachydactyly but normal stature. Probands 3, 4, and 5 (missense variants) presented with isolated brachydactyly but their affected parents have short stature and brachydactyly.

Proband 13 came from a large family with multiple affected individuals. Mild short stature and brachydactyly was present in all the affected individuals except the proband's niece, who at age 15 months has normal stature, although at the lower limit, and no signs of brachydactyly. This incomplete penetrance maybe due to her young age and it is highly likely that she will go on to at least have short stature. Interestingly, the variant identified in this proband, p.(Val298Met), was also observed in proband 14, suggesting a common ancestor.

Three of the patients were born small for gestational age in length but not in weight, in agreement with previous reports (26).

Our findings demonstrate that *IHH* is a good candidate for screening in patients with short stature and variable brachydactyly or other hand-and-feet anomalies in them or their parents. The description of further patients and larger pedigrees as well as the follow-up of these children to adult height will help us in the future to define more precisely the phenotype and the possible correlation genotype-phenotype.

Also, functional studies will be important to validate the VUS. However, these assays are not rapid nor can they be easily implemented in the diagnostic setting. To date, few variants have been characterized. Three of the first variants to be described in BDA1, located in the N-terminal fragment, p.Glu95Lys (E95K), p.Glu131Lys (E131K), and p.Asn100Glu (D100E), were characterized for their autoprocessing, stability, cholesterol modification, palmitoylation, multimer formation, relative alkaline phosphatase induction activity, dissociation assays for Ptc–C-terminal domain, and its binding affinity to heparin (23). The p.Glu95Lys and p.Asn100Glu variants led to a temperature-sensitive and calcium-dependent instability of N-terminal fragment of Indian Hedgehog, which might contribute to an enhanced intracellular degradation of the mutant proteins via the lysosome. All 3 variants affected Hh binding to Ptc, reducing its capacity to induce cellular differentiation.

The mildness, lack of severity or specificity of skeletal findings, and the absence of them in some cases make NGS the ideal method to explore monogenic causes of short stature with minor skeletal defects. But,

it is also equally important to have a detailed clinical examination including anthropometric assessments of the child and parents and radiological assessment of them. Since the implementation of NGS techniques in the study of short stature, we are beginning to identify genetic defects in the milder forms of short stature (6-8, 27).

In summary, thanks to this study together with our previous study (8), we have now described a total of 21 probands with heterozygous *IHH* variants. Some have mild skeletal defects but others could have been classified as nonsyndromic short stature or ISS. What is clear is that none of these 21 probands showed typical features of BDA1. Thus, this detailed clinical examination of individuals with *IHH* variants in this study together with our previous study (8) has broadened the clinical and radiological spectrum of *IHH* variants.

Acknowledgments

The authors would like to thank the families for their participation in this study.

Financial Support: This work was supported in part by the following grants: SAF2015-66831-R and SAF2017-84646-R from the Ministerio de Economía y Empresa (MINECO) (to K.E.H.); Fundación SEEP (to K.E.H and L.S.-M.), PI16-00073 from Carlos III Institute of Health of the Ministry of Economy and Competitiveness (Spain) cofinanced by the European Regional Development Fund (to G.P.N. and A.P.), GV2017/111040 from the Department of Health of the Basque Government (to G.P.N.) and 2013/03236–5 (to A.A.L.J.) from the São Paulo Research Foundation (FAPESP).

Additional Information

Correspondence and Reprint Requests: Karen E. Heath, PhD, MSc, BSc, Institute of Medical & Molecular Genetics (INGEMM); Paseo Castellana 261, 28046 Madrid, Spain. E-mail: karen.heath@salud.madrid.org.

Disclosure Summary: The authors have nothing to disclose. All authors have submitted the International Committee of Medical Journal Editors Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1. 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;**174**(4):R145-R173.
2. Vasques GA, Arnhold IJP, Jorge AAL. Role of the natriuretic peptide system in normal growth and growth disorders. *Horm Res Paediatr*. 2014;**82**(4):222–229.
3. Wang SR, Jacobsen CM, Carmichael H, et al. Heterozygous mutations in natriuretic peptide receptor-B (*NPR2*) gene as a cause of short stature. *Hum Mutat*. 2015;**36**(4):474-481.
4. Hisado-Oliva A, Garre-Vázquez AI, Santaolalla-Caballero F, et al. Heterozygous *NPR2* mutations cause disproportionate short stature, similar to Léri-Weill dyschondrosteosis. *J Clin Endocrinol Metab*. 2015;**100**(8):E1133-E1142.
5. Gkourogianni A, Andrew M, Tyzinski L, et al. Clinical characterization of patients with autosomal dominant short stature due to aggrecan mutations. *J Clin Endocrinol Metab*. 2017;**102**(2):460-469.
6. Sentchordi-Montané L, Aza-Carmona M, Benito-Sanz S, et al. Heterozygous aggrecan variants are associated with short stature and brachydactyly: description of 16 probands and a review of the literature. *Clin Endocrinol (Oxf)*. 2018;**88**(6):820-829.
7. Hisado-Oliva A, Ruzafa-Martin A, Sentchordi L, et al. Mutations in C-natriuretic peptide (NPPC): a novel cause of autosomal dominant short stature. *Genet Med*. 2018;**20**(1):91-97.
8. Vasques GA, Funari MFA, Ferreira FM, et al. *IHH* gene mutations causing short stature with nonspecific skeletal abnormalities and response to growth hormone therapy. *J Clin Endocrinol Metab*. 2018;**103**(2):604-614.
9. Sasai N, Toriyama M, Kondo T. Hedgehog signal and genetic disorders. *Front Genet*. 2019;**10**:1103.
10. Minina E, Wenzel HM, Kreschel C, et al. BMP and Ihh/PTHrP signaling interact to coordinate chondrocyte proliferation and differentiation. *Development*. 2001;**128**(22):4523-4534.
11. Long F, Chung UI, Ohba S, McMahon J, Kronenberg HM, McMahon AP. Ihh signaling is directly required for the osteoblast lineage in the endochondral skeleton. *Development*. 2004;**131**(6):1309-1318.
12. Gao B, Guo J, She C, et al. Mutations in *IHH*, encoding Indian hedgehog, cause brachydactyly type A–1. *Nature Genet*. 2001;**28**(4):386–388.
13. Liu M, Wang X, Cai Z, et al. A novel heterozygous mutation in the Indian hedgehog gene (*IHH*) is associated with brachydactyly type A1 in a Chinese family. *J Hum Genet*. 2006;**51**(8):727-731.
14. Lodder EM, Hoogeboom AJ, Coert JH, de Graaff E. Deletion of 1 amino acid in Indian hedgehog leads to brachydactylyA1. *Am J Med Genet A*. 2008;**146A**(16):2152-2154.
15. Stattin EL, Lindén B, Lönnerholm T, Schuster J, Dahl N. Brachydactyly type A1 associated with unusual radiological findings and a novel Arg158Cys mutation in the Indian hedgehog (*IHH*) gene. *Eur J Med Genet*. 2009;**52**(5):297-302.
16. Jang MA, Kim OH, Kim SW, Ki CS. Identification of p.Glu131Lys mutation in the *IHH* gene in a Korean patient with brachydactyly type A1. *Ann Lab Med*. 2015;**35**(3):387-389.
17. Salian S, Shukla A, Nishimura G, Girisha KM. Severe form of brachydactyly type A1 in a child with a c.298G>A mutation in *IHH* gene. *J Pediatr Genet*. 2017;**6**(3):177-180.
18. Hellemans J, Coucke PJ, Giedion A, et al. Homozygous mutations in *IHH* cause acrocapitofemoral dysplasia, an autosomal recessive disorder with cone-shaped epiphyses in hands and hips. *Am J Hum Genet*. 2003;**72**(4):1040-1046.
19. Byrnes AM, Racacho L, Grimsey A, et al. Brachydactyly A-1 mutations restricted to the central region of the N-terminal active fragment of Indian hedgehog. *Eur J Hum Genet*. 2009;**17**(9):1112-1120.
20. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. 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(5):405-424.
21. Fredriks AM, van Buuren S, van Heel WJ, Dijkman-Neerincx RH, Verloove-Vanhorick SP, Wit JM. Nationwide age references for sitting height, leg length, and sitting height/height ratio, and their diagnostic value for disproportionate growth disorders. *Arch Dis Child*. 2005;90(8):807-812.
22. Malaquias AC, Scalco RC, Fontenele EG, et al. The sitting height/height ratio for age in healthy and short individuals and its potential role in selecting short children for SHOX analysis. *Horm Res Paediatr*. 2013;80(6):449-456.
23. Ma G, Yu J, Xiao Y, et al. Indian hedgehog mutations causing brachydactyly type A1 impair hedgehog signal transduction at multiple levels. *Cell Res*. 2011;21(9):1343-1357.
24. Kirkpatrick TJ, Au KS, Mastrobattista JM, McCready ME, Bulman DE, Northrup H. Identification of a mutation in the Indian hedgehog (*IHH*) gene causing brachydactyly type A1 and evidence for a third locus. *J Med Genet*. 2003;40(1):42-44.
25. Marouli E, Graff M, Medina-Gomez C, et al; EPIC-InterAct Consortium; CHD Exome+ Consortium; ExomeBP Consortium; T2D-Genes Consortium; GoT2D Genes Consortium; Global Lipids Genetics Consortium; ReproGen Consortium; MAGIC Investigators. Rare and low-frequency coding variants alter human adult height. *Nature*. 2017;542(7640):186-190.
26. Freire BL, Homma TK, Funari MFA, et al. Multigene sequencing analysis of children born small for gestational age with isolated short stature. *J Clin Endocrinol Metab*. 2019;104(6):2023-2030.
27. Dauber A. Genetic testing for the child with short stature—has the time come to change our diagnostic paradigm? *J Clin Endocrinol Metab*. 2019;104(7):2766-2769.