IHH Gene Mutations Causing Short Stature With Nonspecific Skeletal Abnormalities and Response to Growth Hormone Therapy

Gabriela A. Vasques,[1] Mariana F. A. Funari,[2] Frederico M. Ferreira,[3] Miriam Aza-Carmona,[4,5,6] Lucia Sentchordi-Montané,[4,5] Jimena Barraza-García,[4,5,6] Antonio M. Lerario,[1,7] Guilherme L. Yamamoto,[8,9] Michel S. Naslavsky,[9] Yeda A. O. Duarte,[10] Debora R. Bertola,[8] Karen E. Heath,[4,5,6] and Alexander A. L. Jorge[1]

[1]Unidade de Endocrinologia Genetica (LIM/25), Hospital das Clinicas da Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil 01246-903; [2]Unidade de Endocrinologia do Desenvolvimento, Laboratorio de Hormonios e Genetica Molecular (LIM/42), Hospital das Clinicas da Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil 05403-010; [3]Laboratorio de Imunologia, Instituto do Coração, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil 05403-900; [4]Institute of Medical and Molecular Genetics, IdiPAZ, Universidad Autónoma de Madrid, Madrid, Spain 28049; [5]Centro de Investigacion Biomedica em Red de Enfermedades Raras, Instituto de Salud Carlos III, Madrid, Spain 28029; [6]Skeletal Dysplasia Multidisciplinary Unit, Hospital Universitario La Paz, Madrid, Spain 28046; [7]Department of Internal Medicine, Division of Metabolism, Endocrinology and Diabetes, University of Michigan, Ann Arbor, Michigan 48109; [8]Unidade de Genetica Clinica, Instituto da Criança do Hospital das Clinicas da Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil 05403-000; [9]Centro de Pesquisa sobre o Genoma Humano e Células Tronco, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil 05508-090; and [10]Departamento de Epidemiologia da Faculdade de Saude Publica, Universidade de São Paulo, São Paulo, Brazil 03178-200

Context: Genetic evaluation has been recognized as an important tool to elucidate the causes of growth disorders.

Objective: To investigate the cause of short stature and to determine the phenotype of patients with *IHH* mutations, including the response to recombinant human growth hormone (rhGH) therapy.

Patients and Methods: We studied 17 families with autosomal-dominant short stature by using whole exome sequencing and screened *IHH* defects in 290 patients with growth disorders. Molecular analyses were performed to evaluate the potential impact of *N*-terminal *IHH* variants.

Results: We identified 10 pathogenic or possibly pathogenic variants in *IHH*, an important regulator of endochondral ossification. Molecular analyses revealed a smaller potential energy of mutated IHH molecules. The allele frequency of rare, predicted to be deleterious *IHH* variants found in short-stature samples (1.6%) was higher than that observed in two control cohorts (0.017% and 0.08%; $P < 0.001$). Identified *IHH* variants segregate with short stature in a dominant inheritance pattern. Affected individuals typically manifest mild disproportional short stature with a frequent finding of shortening of the middle phalanx of the fifth finger. None of them have classic features of brachydactyly type A1, which was previously associated with *IHH* mutations. Five patients heterozygous for *IHH* variants had a good response to rhGH therapy. The mean change in height standard deviation score in 1 year was 0.6.

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

Received 13 September 2017. Accepted 10 November 2017.
First Published Online 15 November 2017

Abbreviations: ABraOM, Brazilian genomic variants; ACMG, American College of Medical Genetics and Genomics; AMP, Association for Molecular Pathology; BDA1, brachydactyly type A1; BMP-V, brachymesophalangia V; CDO, cell adhesion molecule-related, down-regulated by oncogenes; gnomAD, Genome Aggregation Database; Hh, Hedgehog; ISS, idiopathic short stature; MIM, Mendelian Inheritance in Man; rhGH, recombinant human growth hormone; SDS, standard deviation score; SGA, small for gestational age; SH:H, sitting height to height ratio; SHH, Sonic Hedgehog; WES, whole-exome sequencing.

Conclusion: Our study demonstrated the association of pathogenic variants in *IHH* with short stature with nonspecific skeletal abnormalities and established a frequent cause of growth disorder, with a preliminary good response to rhGH. (***J Clin Endocrinol Metab*** **103: 604–614, 2018)**

Most apparently healthy children with short stature are classified as having idiopathic short stature (ISS). The conventional evaluation based on clinical findings complemented by laboratory and image examinations is unable to identify the cause of growth impairment observed in these children. Variation in human height is mostly explained by heritable factors; thus, genetic evaluation could elucidate the causes for growth disorders (1). Common polymorphisms are associated with height in healthy individuals, with a small effect size per locus (2). Alternatively, there is evidence that rare genetic variants are involved with larger effects on stature (3–5). The candidate gene approach has identified defects in a few genes associated with growth impairment observed in children classified as ISS (6). Recently, several whole-exome sequencing (WES) studies have reported gene defects responsible for distinct forms of short stature, including genes expressed in growth plate (7). Heterozygous mutations in *ACAN* have been identified in families with ISS with accelerated bone maturation (8, 9). An *FBN1* mutation was identified in two patients with severe short stature and mild form of acromicric dysplasia (10). Finally, heterozygous mutations in *NPPC* were recently associated with autosomal-dominant short stature and small hands phenotype (11). Hence, WES is a potent tool to screen for candidate genes, identify associations between genes and the phenotype, and broaden the phenotypic spectrum of growth disorders associated with a known gene.

In the current study, we first investigated families with autosomal-dominant short stature through WES. This approach allowed us to identify three families with pathogenic variants in the Indian hedgehog gene [*IHH*, Mendelian Inheritance in Man (MIM) 600726]. This gene codifies an important paracrine regulator of endochondral ossification. Heterozygous mutations in *IHH* are recognized as the cause of brachydactyly type A1 (BDA1; MIM 112500) (12). Because our patients with heterozygous *IHH* variants did not present classic features of this skeletal dysplasia, we have expanded the investigation of *IHH* defects in a large cohort of patients with ISS and patients with mild skeletal dysplasia. Our results have extended the known phenotype of *IHH* defects and revealed that heterozygous *IHH* mutations are a fairly frequent cause of short stature with nonspecific skeletal abnormalities (MIM 616255). Additionally, we have observed a good short response to recombinant human growth hormone (rhGH) in affected patients under therapy.

Patients and Methods

Patients

The respective local ethics committees approved the studies, and the patients or guardians gave their written informed consent. WES was performed on 17 independent Brazilian families with autosomal-dominant ISS as part of an exome sequencing project of patients with growth disorders. All probands fulfilled the following diagnostic criteria: normal birth weight for gestational age, height standard deviation score (SDS) ≤ -2, unremarkable medical history, and absence of abnormal findings on clinical examination or in laboratory tests that could account for short stature. A second Brazilian cohort of children with short stature of unknown cause [111 with ISS and 19 born small for gestational age (SGA) but with family history of short stature] had *IHH* screened by Sanger sequencing (n = 81) or targeted gene sequencing panel (n = 49). Clinical characteristics of the Brazilian cohort are shown in Supplemental Table 1. Additionally, a Spanish cohort of short-stature patients referred for unspecific brachydactyly (n = 60) or mild skeletal dysplasia (n = 100) was evaluated with a skeletal dysplasia–targeted gene sequencing panel that included *IHH*. In all enrolled patients and their relatives, genomic DNA was obtained from peripheral blood leukocytes by using standard techniques. A cohort of 609 Brazilian elderly adults (http://abraom.ib.usp.br/), representative of the healthy population, was analyzed with WES and was used to compare the frequency of *IHH* variants in the Brazilian healthy population.

Exome sequencing

We performed WES in the probands and their first-degree relatives according to previously published protocols (10, 13). Briefly, the libraries were constructed with the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions. The sequences were generated in the HiSEQ 2500 platform (Illumina, San Diego, CA) running on paired-end mode. Reads were aligned to the GRCh37/hg19 assembly of the human genome with the Burrows-Wheeler Alignment (BWA-mem) aligner. Variant calling included single-nucleotide variants and small insertions and deletions and was performed with Freebayes. The resulting data (in variant call format) were annotated with ANNOVAR. The median coverage of the target bases was 114×, with 97% of the target bases having ≥10× coverage.

Targeted sequencing

Patients were analyzed by a customized panel of targeted sequencing, which included *IHH* [for Brazilian patients: Agilent SureSelect assay (Agilent Technologies); for Spanish patients: NimbleGen SeqCap EZ capture (Roche, Pleasanton, CA)]. Sequencing was performed on an Illumina MiSeq or NextSeq platform in paired-end mode. Inhouse bioinformatic analysis was performed. The sequences were aligned to the reference human assembly (GRCh37/hg19). The median coverage of the target bases was 409× and 215×, with 95% and 94.1% of the target bases being covered ≥20× for Brazilian and Spanish patients, respectively.

Data analysis

The exome and the targeted panel sequencing data were screened for rare variants (minor allele frequency $< 0.1\%$ in public and in-house databases) located in exonic regions and consensus splice site sequences. Subsequently, our variant filtration prioritized genes on the basis of their potential to be pathogenic: loss-of-function variants and variants predicted to be pathogenic by multiple *in silico* programs. For variants identified by WES, we selected variants that fitted an autosomal-dominant model in the selected families (Supplemental Figs. 1–3). The sequencing reads carrying candidate variants were inspected visually using the Integrative Genomics Viewer (Broad Institute, Cambridge, MA).

Sanger sequencing

All putative pathogenic variants observed by exome and targeted panel sequencing as well as segregation analysis in the families were validated and genotyped by Sanger sequencing. The primers for *IHH* candidate gene screening were designed to amplify all exons and exon-intron boundaries of the NM_002181.3 transcript (primer sequences and amplification protocols are available upon request). Polymerase chain reaction products were bidirectionally sequenced and analyzed in an ABI 3100/3700XL sequencer (Applied Biosystems, Foster City, CA).

Pathogenicity analysis of identified variants

The pathogenicity of the variants identified during genetic analysis was classified according to the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines (13). To support the interpretation of familial cosegregation, a mathematical quantitative criterion was added to the ACMG/AMP guidelines to allow a statistical quantification of familial aggregation data (14). Population data criteria were evaluated by using two public genomic databases: Genome Aggregation Database (gnomAD: http://gnomad.broadinstitute.org/) and Brazilian genomic variants (ABraOM: http://abraom.ib.usp.br/). Several different computational tools were used to obtain predictive data of each variant (Supplemental Table 2). Because the crystal structure of the *N*-terminal domain of human IHH had been resolved, the potential impact of the mutations located in this region (Table 1) was further evaluated by molecular simulations carried out with the human IHH molecular model and its mutated versions (Supplemental Methods).

To provide additional genetic evidence of the association of *IHH* with short-stature phenotype with nonspecific skeletal findings (15), we performed aggregate variant analyses comparing allele frequencies between our cohorts of patients and public databases (gnomAD and ABraOM). In these databases, we selected variants with similar characteristics of the *IHH* variants observed in our cohort of short stature patients: rare variants (minor allele frequency < 0.01) with loss-of-function effect (stop gain, frameshift, or consensus splicing site variants) or rare nonsynonymous variants predicted to be pathogenic by at least four *in silico* tools (PROVEAN, SIFT, PolyPhen-2, and Mutation Assessor). Allele frequency differences between groups were analyzed by χ^2 test, and statistical significance was set at $P < 0.05$. Statistical analyses were performed by using the SIGMAstat statistical software package (Windows version 4.0; SPSS Inc., San Rafael, CA).

Results

Genetic analysis

Three different heterozygous *IHH* variants were identified in 3 of 17 families with ISS individuals analyzed through WES (Fig. 1A–1C; Supplemental Figs. 1–3). Because the affected individuals did not present specific signs of a skeletal dysplasia (including BDA1), we screened 130 children with short stature of unknown cause for rare variants in *IHH*. In this group, another two heterozygous *IHH* variants were identified (Fig. 1D and 1E). Among a cohort of Spanish patients with short stature and

Table 1. *In Silico* Analysis and ACMG/AMP Classification of *IHH* Identified Variants

IHH cDNA Variant[a] (Protein)	Domain	SIFT	Polyphen	Mutation Assessor	PROVEAN	CADD V1.3	GERP ++	ACMG-AMP Classification
Identified in Brazilian patients								
c.172G>A (p.Glu58Lys)	N-terminal signaling domain	0/D	1.0/D	3.085/M	−3.03/D	33.0	4.22	Pathogenic
c.446G>A (p.Arg149His)	N-terminal signaling domain	0.01/D	1.0/D	3.085/M	−4.63/D	35.0	5.78	Likely pathogenic
c.532G>A (p.Val178Met)	N-terminal signaling domain	0.02/D	0.99/D	3.085/M	−2.77/D	32.0	5.68	Likely pathogenic
c.532G>C (p.Val178Leu)	N-terminal signaling domain	0/D	0.699/P	3.085/M	−2.77/D	24.7	5.68	Pathogenic
c.1139G>T (p.Gly380Val)	C-terminal domain	0.04/D	1.0/D	3.235/M	−5.87/D	29.0	4.64	Likely pathogenic
Identified in Spanish patients								
c.319delT (p.Cys107Alafs*5)	N-terminal signaling domain	NA	NA	NA	NA	NA	NA	Pathogenic
c.531G>C (p.Trp177Cys)	N-terminal signaling domain	0/D	1.0/D	3.075/M	−11.96/D	25.9	5.68	Likely pathogenic
c.797dupC (p.Arg267Thrfs*15)	C-terminal domain	NA	NA	NA	NA	NA	NA	Pathogenic
c.949G>A (p.Val317Met)	C-terminal domain	0.01/D	0.998/D	3.145/M	−2.12/N	26.3	5.16	Likely pathogenic
c.1202T>C (p.Phe401Ser)	C-terminal domain	0.009/D	0.99/D	2.470/M	−2.78/D	28.9	4.64	Likely pathogenic

ACMG/AMP classification (13, 14).

Abbreviations: cDNA, complementary DNA; NA, not available.

[a]Gene transcript ID: NM_002181.3, Gene:ENSG00000163501, transcript: ENST00000295731.

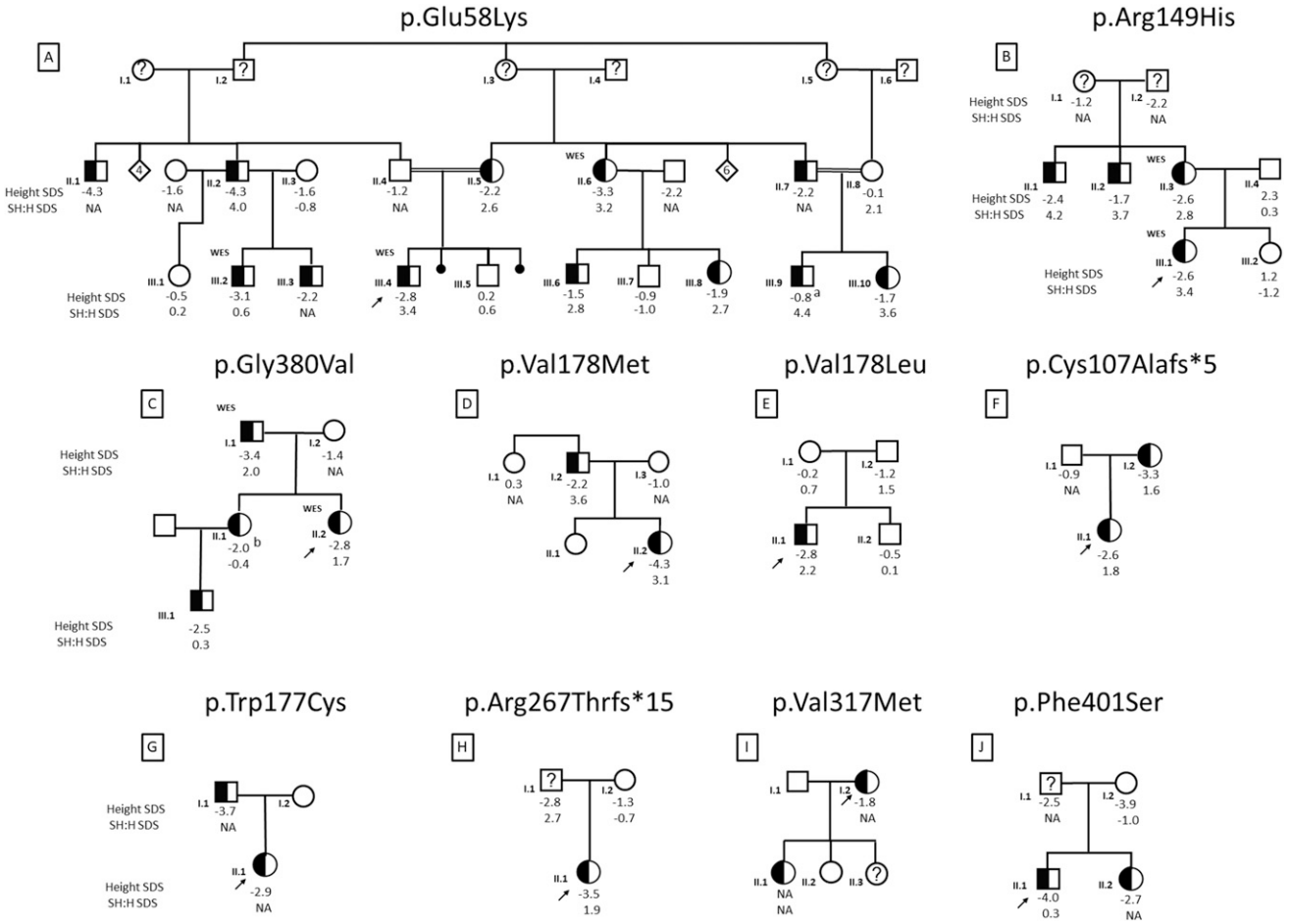

Figure 1. Pedigrees of the affected families carrying heterozygous *IHH* mutations. (A–E) Brazilian families with ISS. (F–J) Spanish families with mild skeletal dysplasia or brachydatyly. The arrows indicate the probands, and WES indicates individuals who underwent WES. Individuals with heterozygous *IHH* mutations are indicated by half-filled symbols, whereas individuals homozygous for *IHH* wild-type allele are indicated as open symbols. Question marks indicate an unknown genotype. Height SDS and SH:H SDS at first medical visit are shown below the symbols. Abbreviation: NA, no data available. [a]Adult height SDS of −1.9; [b]previous treatment with rhGH.

mild skeletal dysplasia or brachydactyly (n = 160), five additional heterozygous *IHH* variants were identified by targeted sequencing (Fig. 1F–1J). Thus, a total of 10 heterozygous *IHH* variants were identified, 2 frameshift mutations resulting in the premature termination of the protein, and 8 missense variants (Table 1). All of them are predicted to be pathogenic by multiple *in silico* programs. Six of the variants are located in the *N*-terminal domain [p.Glu58Lys (c.172G>A), p.Arg149His (c.446G>A), p.Cys107Alafs*5 (c.319delT), p.Trp177Cys (c.531G>C), p.Val178Leu (c.532G>C), p.Val178Met (c.532G>A)], and four are located in the C-terminal domain [p.Arg267Thrfs*15 (c.797dupC), p.Val317Met (c.949G>A), p.Gly380Val (c.1139G>T), p.Phe401Ser (c.1202T>C)]. Nine of the variants are absent in public databases, whereas the p.Val317Met variant is present at an extremely low allelic frequency (1/241,680) in gnomAD. Eight of the variants were confirmed to segregate with the autosomal-dominant short-stature phenotype (Fig. 1). One variant was *de novo* (paternity and maternity confirmed). Thus, after applying the ACMG-AMP guidelines, four variants were classified as pathogenic and six as likely pathogenic (Table 1).

Primary sequence analyses showed that all IHH-mutated amino acid residues are extremely conserved among non-redundant homologous and related sequences. Although some hydrogen bonds were locally disrupted near the mutated amino acid residues, in general, the presence of the mutations provided more molecular contacts for the whole molecules (Supplemental Fig. 4). In addition, results of the IHH molecular simulations revealed that IHH models bearing *N*-terminal mutations presented a smaller mean potential energy compared with that of the native molecules (Supplemental Table 3; Supplemental Fig. 5). Conversely, except for the IHH Glu58Lys and Arg149His molecules, the binding energies of the atoms Zn412, Ca413 and Ca414 to all others mutated molecules are greater than that of the wild type one (Supplemental Table 3).

The allele frequency of rare *IHH* variants predicted to be deleterious found in both cohort of patients with short

stature (1.7% and 1.56% for Brazilian and Spanish cohorts, respectively) was expressively higher than that observed in individuals from gnomAD (0.017%; $P < 0.001$) (Table 2). Considering only the Brazilian cohort, we also observed a significantly higher frequency of rare *IHH* variants predicted to be deleterious in short-stature individuals (1.7%) in comparison with the ABraOM database [1/1218 alleles (0.08%); $P < 0.001$] (Table 2; Supplemental Fig. 6). Furthermore, the allele frequency of *IHH* variants was similar in Brazilian children with [4/214 alleles (1.9%)] or without [1/80 alleles (1.3%)] familial short stature.

Clinical phenotypes

The phenotypic characteristics of the probands and other children with heterozygous *IHH* variants are shown in Table 3. Four of the 12 Brazilian children were born SGA regarding birth length, and the other 8 had a birth length SDS in the lower part of the normal range (mean birth length, −1.4 ± 0.09 SDS; range, −2.7 to −0.1 SDS). The mean height SDS of the Brazilian children was −2.6 ± 0.9 (range, −4.3 to −0.8 SDS). The affected patient (AIII.9) with a height SDS of −0.8 (Fig.1A) was a 14.6-year-old boy with advanced bone age (16.5 years), and his adult height SDS is −1.9. Most affected Brazilian children (n = 8/12) presented mild disproportionate short stature [mean sitting height to height ratio (SH:H) SDS, 2.4 ± 1.2; range, 0.3 to 3.6]. Most affected children had mild delayed bone age, but we observed two of them with advanced bone age (Table 3 individuals AIII.6 and AIII.9). Abnormal hand radiographs were observed in 6 of 12 Brazilian children. Two children presented only shortening of the middle phalanx of the fifth finger. Other radiological hand findings include varying degrees of shortening of the middle phalanx of the second and fifth fingers with cone-shaped epiphyses (Fig. 2A; Supplemental Fig. 7). Foot radiographs of patients with heterozygous *IHH* mutations showed a recurrent shortening of the distal phalanx of the hallux. Other radiological findings observed in feet were cone-shaped epiphyses of proximal phalanges of the second, third, fourth, and fifth toes (Fig. 2B; Supplemental Fig. 8).

All five Spanish cases were detected in a cohort of patients with mild skeletal dysplasia (3/100) or suspected to have brachydactyly (2/60). Low birth length (−2.9 SDS) was observed in one patient (GII.1). The height SDS of Spanish children ranged from −4.0 to −2.6 SDS (mean, −3.2 ± 0.6 SDS). SH:H SDS values were in the upper part of the normal range (1.8 and 1.9 SDS) in two of three available probands. Patient JII.1 had a severe delay (−3.8 years) in bone age with respect to chronological age. All five patients had mild abnormal skeletal surveys, typically shortening of middle phalanx of the second and fifth fingers (Supplemental Fig. 7).

A total of 14 adult individuals heterozygous for *IHH* variants could be assessed (10 Brazilian and 4 Spanish). Their mean height SDS was −3.0±1.1 (range, −4.3 to −1.7). Eight of nine affected adult individuals available have altered body proportion (SH:H SDS ranged from 2.0 to 4.2) (Supplemental Table 4).

Growth response to rhGH treatment

Five patients, all from the Brazilian cohort, with heterozygous *IHH* variants have been treated with rhGH therapy (50 μg/kg/d) (Table 4). The onset of treatment was prepuberty in all of them. One girl had started puberty during the first year of rhGH treatment (patient CII.2). After exclusion of this patient from first-year growth response, the four remaining patients had a mean improvement in height SDS of 0.6 (range, 0.46 to 0.79) and their height velocity ranged from 8.0 to 10.8 cm/y.

Discussion

Longitudinal bone growth is essential to determine adult body height and depends on the growth plate chondrogenesis, a complex process regulated by endocrine and paracrine factors. Over the past years, mutations in different genes involved in the growth plate have been

Table 2. Comparison of Allele Frequency for Rare and Potential Deleterious Alleles Between Patients Selected for Short Stature and Public Databases

Variable	Potential Deleterious Alleles[a]	Total No. of Analyzed Alleles	Allele Frequency (%)
gnomAD	40	236,524	0.017
ABraOM	1	1218	0.082
Brazilian cohort of children with short stature of unknown cause	5	294	1.70[b]
Spanish cohort of patients with mild skeletal dysplasia	5	320	1.56[a]

[a]Rare variants (MAF of 0.01) with loss-of-function effect (stop gain, frameshift, or consensus splicing site variants) or rare nonsynonymous variants predicted to be pathogenic by at least four *in silico* tools (PROVEAN, SIFT, PolyPhen-2 and Mutation Assessor).

[b]$P < 0.001$ for comparison with gnomAD or ABraOM.

Table 3. Clinical Phenotypes of Probands and Their Child Family Members With Heterozygous *IHH* Mutations

Patient ID	*IHH* Mutations	Sex	Birth Length SDS	Age (y)	Bone Age (y)	Height SDS	SH:H SDS	Hand Radiographic Findings
Brazilian children								
AIII.2	p.Glu58Lys	M	−2.1	5.6	5.0	−3.1	0.6	Cone-shaped epiphyses of middle phalanx of fifth finger and of distal phalanx of thumb
AIII.3	p.Glu58Lys	M	−0.8	1.5	NA	−2.2	NA	NA
AIII.4	p.Glu58Lys	M	−2.7	3.4	2.7	−2.8	3.4	Cone-shaped epiphyses of middle phalanx of second and fifth fingers and of distal phalanx of thumb
AIII.6	p.Glu58Lys	M	−1.6	8.9	9.4	−1.5	2.8	No abnormal findings
AIII.8	p.Glu58Lys	F	−1.9	3.5	2.7	−1.9	2.7	Shortening of middle phalanx of fifth finger
AIII.9	p.Glu58Lys	M	−0.1	14.6	16.5	−0.8[a]	4.4	No abnormal findings (adult bone age)
AIII.10	p.Glu58Lys	F	−0.7	7.4	6.8	−1.7	3.6	Cone-shaped epiphysis of middle phalanx of fifth finger
BIII.1	p.Arg149His	F	−2.4	8.0	6.8	−2.6	3.4	Shortening of middle phalanx of second and fifth fingers with cone-shaped epiphyses
CII.2	p.Gly380Val	F	−0.2	9.2	7.9	−2.8	1.7	No abnormal findings
CIII.1	p.Gly380Val	M	−1.1	1.5	NA	−2.5	0.3	No abnormal findings
DII.2	p.Val178Met	F	−2.3	9.4	5.0	−4.3	3.1	Shortening of middle phalanx of fifth finger
EII.1	p.Val178Leu	M	−1.0	7.1	5.8	−2.8	2.2	No abnormal findings
Spanish individuals								
FII.1	p.Cys107Alafs*5	F	NA	13	13	−2.6	1.8	Braquimetacarpal with shortening of middle phalanx of second and fifth fingers
GII.1	p.Trp177Cys	F	−2.9	16	NA	−2.9	NA	Severe shortening of middle phalanx of second and fifth fingers with cone-shaped epiphyses
HII.1	p.Arg267Thrfs*15	F	NA	7	6.0	−3.5	1.9	Severe shortening of middle phalanx of second and fifth fingers
II.2	p.Val317Met	F	NA	Adult	NA	−1.8	NA	Severe shortening of middle phalanx of fifth finger and slight shortening of middle phalanx of second finger
JII.1	p.Phe401Ser	M	−0.4	7.8	4.0	−4.0	0.3	No abnormal findings

Birth length and height SDS are according to population curves.

Abbreviations: F, female; M, male; NA, data not available.

[a]During follow-up, the patient had a growth velocity of 0.6 cm/y, and his height SDS for adult age is −1.9.

implicated in the etiology of several cases of skeletal dysplasia, a condition frequently presented with marked growth impairment (6, 16). Furthermore, a limited number of genes implicated in severe skeletal dysplasia [*SHOX* (17), *NPR2* (18), *ACAN* (8), *FBN1* (10), *NPPC* (11)] have been associated with nonsyndromic short stature, which is commonly classified as ISS in clinical practice.

In the current study, we identified heterozygous *IHH* mutations in 5 of 147 children initially classified as ISS. Additionally, another 5 heterozygous *IHH* mutations were identified among short stature patients with subtle signs of a skeletal dysplasia (3/100) or with mild brachydactyly (2/60). According to ACMG-AMP criteria (13), the identified *IHH* variants were classified as pathogenic or likely pathogenic (Table 1).

Along with IHH, Hedgehog (Hh) family proteins include other two Hh homologs with high level of sequence conservation: Sonic Hh (SHH) and Desert Hh, essential for the nervous system and testis development, respectively (19). Hh proteins are synthesized in the endoplasmic reticulum as a precursor that undergoes an autocatalytic process to yield the *N*-terminal domain, which has biological signaling activities (20). Hh action is mediated through Patched and Smoothened receptors and is positively regulated by cell adhesion molecule-related, downregulated by oncogenes (CDO) and brother of CDO in a calcium-dependent binding mode (19). *IHH* is expressed in the prehypertrophic chondrocytes of cartilage and coordinates proliferation and differentiation of chondrocytes during endochondral bone development (21). Homozygous *IHH* mutations cause acrocapitofemoral dysplasia (MIM 607778), a disorder characterized by severe disproportionate short stature with cone-shaped epiphyses in hands and hips (22). In addition, heterozygous mutations in *IHH* cause BDA1 (MIM 112500). BDA1 is characterized by a marked shortening of the middle phalanges, which are frequently rudimentary or fused with the terminal phalanges. The proximal phalanges of the thumbs and big toes are also short (12). Interestingly, short stature was not consistently demonstrated in patients with BDA1 (12, 23).

The variants identified in the current study were located throughout the *IHH*, including two frameshift mutations and eight missense mutations. These findings are in contrast with previous variants associated with BDA1, which were all missense and restricted to the central region of the *N*-terminal active fragment of IHH

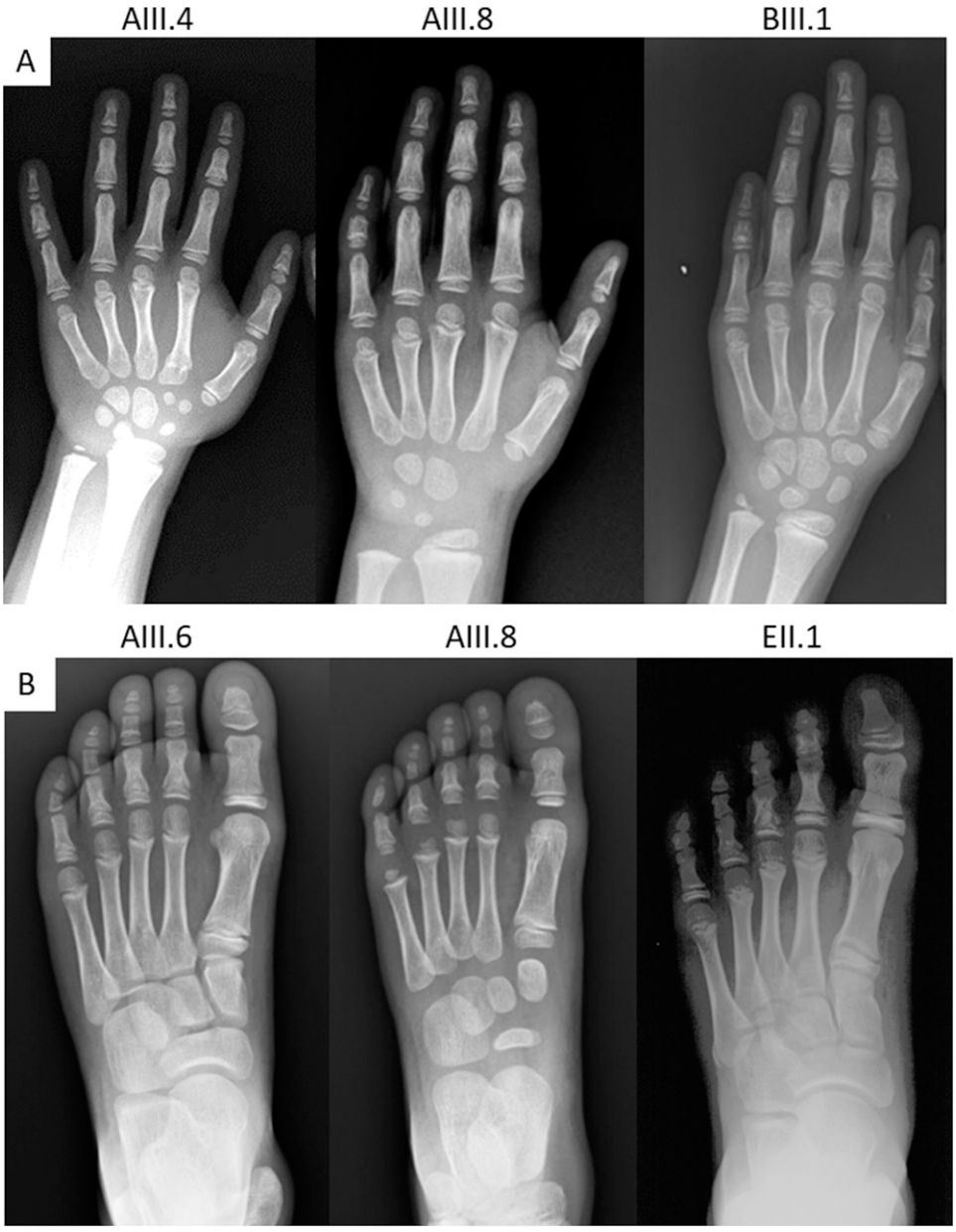

Figure 2. Hand and feet radiographs of children carrying heterozygous *IHH* mutations. (A) Hand radiographs show varying degrees of shortening of the middle phalanx of the second and fifth fingers with cone-shaped epiphyses. (B) Foot radiographs show shortening of distal phalanx of the hallux (individuals AIII.6 and AIII.8) and cone-shaped epiphyses of proximal phalanges.

(24) (Supplemental Fig. 9). Similar to our findings, variants in *SHH* associated to holoprosencephaly (missense, nonsense, or frameshift) are located throughout the gene (25). In addition, after single–amino acid code alignment of IHH and SHH, we observed that two of the *IHH* mutations identified in our patients (p.Glu58Lys and p.Arg149His) are located in the identical residues of pathogenic *SHH* mutations (p.Glu53Lys and

Table 4. Response to rhGH Therapy in Patients With Heterozygous *IHH* Variants

Patient ID	*IHH* Mutations	Sex	Age at Initiation of rhGH (y)	Height SDS Before rhGH	Height SDS at 1 y of rhGH	Height Velocity after 1 y of rhGH (cm/y)	Duration of rhGH Therapy (y)	Last Available Height SDS	Total Height SDS Change
AIII.2	p.Glu58Lys	M	6.6	−3.0	−2.6	8.0	1.0	−2.6	0.4
AIII.6	p.Glu58Lys	M	10	−1.5	−1.0	8.0	1.0	−1.0	0.5
BIII.1	p.Arg149His	F	8	−2.6	−1.9	8.1	3.0	−1.8	0.8
EII.1	p.Val178Leu	M	7.1	−2.8	−2.0	10.8	4.7	−0.4	2.4
CII.2	p.Gly380Val	F	10.4	−2.6	−1.7	11.2	4.0	−1.5	1.1

Abbreviations: F, female; M, male.

p.Arg144Pro, respectively) (26). The results of the structural analyses and molecular mechanics simulations experiments based on the IHH molecular structure showed that all mutations located at *N*-terminal domain could affect the structure and, therefore, the biological function of the molecule. The presence of these mutations determines a smaller total mean potential energy that is associated with a more stable molecule, affecting the calcium and zinc cofactor-binding energy (Supplemental Table 3). A greater calcium-binding energies observed in four mutated IHH molecules (p.Glu58Lys, p.Val178Leu, p.Val178Met, and p.Trp177Cys) could affect the interaction to binding partners CDO and brother of CDO, a mechanism already described to *IHH* mutations associated to BDA1 (19).

It is a challenge to determine the functional impact of variants located in the C-terminal domain of Hh proteins. Nevertheless, one study found no difference in phenotype severity between individuals with holoprosencephaly caused by *SHH* mutations located in the N- or C-terminal domains (27). It is postulated that C-terminal variants could impair the preprotein maturation and autocleavage process (26). Further studies are necessary to elucidate molecular mechanisms of *IHH* variants responsible for nonsyndromic short stature.

We provide several lines of genetic evidence that the identified *IHH* variants are associated with short stature with nonspecific skeletal abnormalities (15). First, at each case level, we demonstrated in several families a segregation with growth impairment phenotype in a dominant inheritance pattern (Fig. 1). Additionally, a *de novo* variant (both paternity and maternity confirmed) was identified in a patient with short stature and normal stature parents (Fig. 1E). Second, we provide statistical evidence that rare *IHH* variants are enriched in the two analyzed cohorts of short-stature patients in comparison with public databases that involve a large number of individuals not selected by stature (Table 2; Supplemental Fig. 6).

Patients described in the current study typically manifest mild disproportional short stature. It seems that disproportionality has become more pronounced over the years during childhood. Four probands were born SGA regarding birth length. Intrauterine growth impairment with normal birth weight, especially in children with familial short stature, may suggest defects in genes that play a role in bone elongation. There is a variable phenotype considering the severity of short stature and radiological findings, even among individuals within the same family. The most recurrent radiological finding observed in our patients was varying degrees of shortening of the middle phalanx of the fifth finger, a defining feature of brachymesophalangia V (BMP-V) (28). BMP-V is the most common skeletal anomaly of the hand and can be observed alone (clinically known as brachydactyly type A3) or in association to other skeletal abnormalities and developmental syndromes. Epiphyses of the fifth finger may be associated with a susceptibility to a chondrogenesis disorder because they are among the last to be ossified in the hand. BMP-V was observed in 64.3% (9/14) hand radiographs from individuals heterozygous from *IHH* variants in our cohort, whereas the frequency of BMP-V observed in a population study was 12.1% (165/1360; $P < 0.001$) (28).

The response to rhGH therapy could be assessed in five patients heterozygous for *IHH* variants (Table 4). We considered the short-term response to rhGH therapy presented by this small group of children to be good. The improvement in height SDS at 1 year of rhGH therapy was similar to or better than that previously reported in two larger studies that evaluated responsiveness to rhGH in children with ISS (0.60 SDS and 0.49 SDS, respectively) (29, 30). A different study developed criteria based on height velocity targets for first-year responses in ISS children. Those researchers proposed that height velocity below the mean −1 SD on their plots should be considered a "poor" response. None of our patients had a first-year height velocity below the mean −1 standard deviation; two were plotted above the mean and can be considered as good (31).

In conclusion, our data demonstrate the association of rare pathogenic variants in *IHH* with short stature with nonspecific skeletal abnormalities (MIM 616255). Given the subtle and low specific clinical and radiological findings observed in our patients, only a broad molecular study allowed this association. Thus, we have expanded the phenotype associated with heterozygous *IHH* mutations and established a frequent cause of growth disorder, with a preliminary good response to rhGH.

Acknowledgments

Financial Support: This work was supported by Grants 2013/03236–5 (to A.A.L.J.) and 2013/02162-8 (Laboratório de Sequenciamento em Larga Escala) from the São Paulo Research Foundation; Grants 301871/2016-7 and 459845/2014-4 (to A.A.L.J.) from the National Council for Scientific and Technological Development; Grants SAF2012-30871 and SAF2015-66831-R from El Ministerio de Economía, Industria y Competitividad (to K.E.H.); and an award from the Foundation of the Spanish Society of Pediatric Endocrinology (to L.S.-M. and K.E.H.).

Correspondence and Reprint Requests: Alexander A. L. Jorge, MD, PhD, Faculdade de Medicina da USP (LIM-25), Av. Dr. Arnaldo, 455 5° andar sala 5340, CEP 01246-903 Sao Paulo, Brazil. E-mail: alexj@usp.br.

Disclosure Summary: The authors have nothing to disclose.

References

1. Dauber A, Rosenfeld RG, Hirschhorn JN. Genetic evaluation of short stature. *J Clin Endocrinol Metab*. 2014;**99**(9):3080–3092.
2. Wood AR, Esko T, Yang J, Vedantam S, Pers TH, Gustafsson S, Chu AY, Estrada K, Luan J, Kutalik Z, Amin N, Buchkovich ML, Croteau-Chonka DC, Day FR, Duan Y, Fall T, Fehrmann R, Ferreira T, Jackson AU, Karjalainen J, Lo KS, Locke AE, Mägi R, Mihailov E, Porcu E, Randall JC, Scherag A, Vinkhuyzen AA, Westra HJ, Winkler TW, Workalemahu T, Zhao JH, Absher D, Albrecht E, Anderson D, Baron J, Beekman M, Demirkan A, Ehret GB, Feenstra B, Feitosa MF, Fischer K, Fraser RM, Goel A, Gong J, Justice AE, Kanoni S, Kleber ME, Kristiansson K, Lim U, Lotay V, Lui JC, Mangino M, Mateo Leach I, Medina-Gomez C, Nalls MA, Nyholt DR, Palmer CD, Pasko D, Pechlivanis S, Prokopenko I, Ried JS, Ripke S, Shungin D, Stancáková A, Strawbridge RJ, Sung YJ, Tanaka T, Teumer A, Trompet S, van der Laan SW, van Setten J, Van Vliet-Ostaptchouk JV, Wang Z, Yengo L, Zhang W, Afzal U, Arnlöv J, Arscott GM, Bandinelli S, Barrett A, Bellis C, Bennett AJ, Berne C, Blüher M, Bolton JL, Böttcher Y, Boyd HA, Bruinenberg M, Buckley BM, Buyske S, Caspersen IH, Chines PS, Clarke R, Claudi-Boehm S, Cooper M, Daw EW, De Jong PA, Deelen J, Delgado G, Denny JC, Dhonukshe-Rutten R, Dimitriou M, Doney AS, Dörr M, Eklund N, Eury E, Folkersen L, Garcia ME, Geller F, Giedraitis V, Go AS, Grallert H, Grammer TB, Gräßler J, Grönberg H, de Groot LC, Groves CJ, Haessler J, Hall P, Haller T, Hallmans G, Hannemann A, Hartman CA, Hassinen M, Hayward C, Heard-Costa NL, Helmer Q, Hemani G, Henders AK, Hillege HL, Hlatky MA, Hoffmann W, Hoffmann P, Holmen O, Houwing-Duistermaat JJ, Illig T, Isaacs A, James AL, Jeff J, Johansen B, Johansson Å, Jolley J, Juliusdottir T, Junttila J, Kho AN, Kinnunen L, Klopp N, Kocher T, Kratzer W, Lichtner P, Lind L, Lindström J, Lobbens S, Lorentzon M, Lu Y, Lyssenko V, Magnusson PK, Mahajan A, Maillard M, McArdle WL, McKenzie CA, McLachlan S, McLaren PJ, Menni C, Merger S, Milani L, Moayyeri A, Monda KL, Morken MA, Müller G, Müller-Nurasyid M, Musk AW, Narisu N, Nauck M, Nolte IM, Nöthen MM, Oozageer L, Pilz S, Rayner NW, Renstrom F, Robertson NR, Rose LM, Roussel R, Sanna S, Scharnagl H, Scholtens S, Schumacher FR, Schunkert H, Scott RA, Sehmi J, Seufferlein T, Shi J, Silventoinen K, Smit JH, Smith AV, Smolonska J, Stanton AV, Stirrups K, Stott DJ, Stringham HM, Sundström J, Swertz MA, Syvänen AC, Tayo BO, Thorleifsson G, Tyrer JP, van Dijk S, van Schoor NM, van der Velde N, van Heemst D, van Oort FV, Vermeulen SH, Verweij N, Vonk JM, Waite LL, Waldenberger M, Wennauer R, Wilkens LR, Willenborg C, Wilsgaard T, Wojczynski MK, Wong A, Wright AF, Zhang Q, Arveiler D, Bakker SJ, Beilby J, Bergman RN, Bergmann S, Biffar R, Blangero J, Boomsma DI, Bornstein SR, Bovet P, Brambilla P, Brown MJ, Campbell H, Caulfield MJ, Chakravarti A, Collins R, Collins FS, Crawford DC, Cupples LA, Danesh J, de Faire U, den Ruijter HM, Erbel R, Erdmann J, Eriksson JG, Farrall M, Ferrannini E, Ferrières J, Ford I, Forouhi NG, Forrester T, Gansevoort RT, Gejman PV, Gieger C, Golay A, Gottesman O, Gudnason V, Gyllensten U, Haas DW, Hall AS, Harris TB, Hattersley AT, Heath AC, Hengstenberg C, Hicks AA, Hindorff LA, Hingorani AD, Hofman A, Hovingh GK, Humphries SE, Hunt SC, Hypponen E, Jacobs KB, Jarvelin MR, Jousilahti P, Jula AM, Kaprio J, Kastelein JJ, Kayser M, Kee F, Keinanen-Kiukaanniemi SM, Kiemeney LA, Kooner JS, Kooperberg C, Koskinen S, Kovacs P, Kraja AT, Kumari M, Kuusisto J, Lakka TA, Langenberg C, Le Marchand L, Lehtimäki T, Lupoli S, Madden PA, Männistö S, Manunta P, Marette A, Matise TC, McKnight B, Meitinger T, Moll FL, Montgomery GW, Morris AD, Morris AP, Murray JC, Nelis M, Ohlsson C, Oldehinkel AJ, Ong KK, Ouwehand WH, Pasterkamp G, Peters A, Pramstaller PP, Price JF, Qi L, Raitakari OT, Rankinen T, Rao DC, Rice TK, Ritchie M, Rudan I, Salomaa V, Samani NJ, Saramies J, Sarzynski MA, Schwarz PE, Sebert S, Sever P, Shuldiner AR, Sinisalo J, Steinthorsdottir V, Stolk RP, Tardif JC, Tönjes A, Tremblay A, Tremoli E, Virtamo J, Vohl MC, Amouyel P, Asselbergs FW, Assimes TL, Bochud M, Boehm BO, Boerwinkle E, Bottinger EP, Bouchard C, Cauchi S, Chambers JC, Chanock SJ, Cooper RS, de Bakker PI, Dedoussis G, Ferrucci L, Franks PW, Froguel P, Groop LC, Haiman CA, Hamsten A, Hayes MG, Hui J, Hunter DJ, Hveem K, Jukema JW, Kaplan RC, Kivimaki M, Kuh D, Laakso M, Liu Y, Martin NG, März W, Melbye M, Moebus S, Munroe PB, Njølstad I, Oostra BA, Palmer CN, Pedersen NL, Perola M, Pérusse L, Peters U, Powell JE, Power C, Quertermous T, Rauramaa R, Reinmaa E, Ridker PM, Rivadeneira F, Rotter JI, Saaristo TE, Saleheen D, Schlessinger D, Slagboom PE, Snieder H, Spector TD, Strauch K, Stumvoll M, Tuomilehto J, Uusitupa M, van der Harst P, Völzke H, Walker M, Wareham NJ, Watkins H, Wichmann HE, Wilson JF, Zanen P, Deloukas P, Heid IM, Lindgren CM, Mohlke KL, Speliotes EK, Thorsteinsdottir U, Barroso I, Fox CS, North KE, Strachan DP, Beckmann JS, Berndt SI, Boehnke M, Borecki IB, McCarthy MI, Metspalu A, Stefansson K, Uitterlinden AG, van Duijn CM, Franke L, Willer CJ, Price AL, Lettre G, Loos RJ, Weedon MN, Ingelsson E, O'Connell JR, Abecasis GR, Chasman DI, Goddard ME, Visscher PM, Hirschhorn JN, Frayling TMElectronic Medical Records and Genomics (eMEMERGEGE) ConsortiumMIGen ConsortiumPAGEGE ConsortiumLifeLines Cohort Study. Defining the role of common variation in the genomic and biological architecture of adult human height. *Nat Genet*. 2014;**46**(11):1173–1186.
3. Zoledziewska M, Sidore C, Chiang CWK, Sanna S, Mulas A, Steri M, Busonero F, Marcus JH, Marongiu M, Maschio A, Ortega Del Vecchyo D, Floris M, Meloni A, Delitala A, Concas MP, Murgia F, Biino G, Vaccargiu S, Nagaraja R, Lohmueller KE, Timpson NJ, Soranzo N, Tachmazidou I, Dedoussis G, Zeggini E, Uzzau S, Jones C, Lyons R, Angius A, Abecasis GR, Novembre J, Schlessinger D, Cucca FUK10K consortiumUnderstanding Society Scientific Group. Height-reducing variants and selection for short stature in Sardinia. *Nat Genet*. 2015;**47**(11):1352–1356.
4. Scalco RC, Hwa V, Domené HM, Jasper HG, Belgorosky A, Marino R, Pereira AM, Tonelli CA, Wit JM, Rosenfeld RG, Jorge AA. STAT5B mutations in heterozygous state have negative impact on height: another clue in human stature heritability. *Eur J Endocrinol*. 2015;**173**(3):291–296.
5. Marouli E, Graff M, Medina-Gomez C, Lo KS, Wood AR, Kjaer TR, Fine RS, Lu Y, Schurmann C, Highland HM, Rüeger S, Thorleifsson G, Justice AE, Lamparter D, Stirrups KE, Turcot V, Young KL, Winkler TW, Esko T, Karaderi T, Locke AE, Masca NG, Ng MC, Mudgal P, Rivas MA, Vedantam S, Mahajan A, Guo X, Abecasis G, Aben KK, Adair LS, Alam DS, Albrecht E, Allin KH, Allison M, Amouyel P, Appel EV, Arveiler D, Asselbergs FW, Auer PL, Balkau B, Banas B, Bang LE, Benn M, Bergmann S, Bielak LF, Blüher M, Boeing H, Boerwinkle E, Böger CA, Bonnycastle LL, Bork-Jensen J, Bots ML, Bottinger EP, Bowden DW, Brandslund I, Breen G, Brilliant MH, Broer L, Burt AA, Butterworth AS, Carey DJ, Caulfield MJ, Chambers JC, Chasman DI, Chen YI, Chowdhury R, Christensen C, Chu AY, Cocca M, Collins FS, Cook JP, Corley J, Galbany JC, Cox AJ, Cuellar-Partida G, Danesh J, Davies G, de Bakker PI, de Borst GJ, de Denus S, de Groot MC, de Mutsert R, Deary IJ, Dedoussis G, Demerath EW, den Hollander AI, Dennis JG, Di Angelantonio E, Drenos F, Du M, Dunning AM, Easton DF, Ebeling T, Edwards TL, Ellinor PT, Elliott P, Evangelou E, Farmaki AE, Faul JD, Feitosa MF, Feng S, Ferrannini E, Ferrario MM, Ferrieres J, Florez JC, Ford I, Fornage M, Franks PW, Frikke-Schmidt R, Galesloot TE, Gan W, Gandin I, Gasparini P, Giedraitis V, Giri A, Girotto G, Gordon SD, Gordon-Larsen P, Gorski M, Grarup N, Grove ML, Gudnason V, Gustafsson S, Hansen T, Harris KM, Harris TB, Hattersley AT, Hayward C, He L, Heid IM, Heikkilä K, Helgeland Ø, Hernesniemi J, Hewitt AW, Hocking LJ, Hollensted M, Holmen OL, Hovingh GK, Howson JM, Hoyng CB, Huang PL, Hveem K, Ikram MA, Ingelsson E, Jackson AU, Jansson JH, Jarvik GP, Jensen GB, Jhun MA, Jia Y, Jiang X, Johansson S, Jørgensen ME, Jørgensen T, Jousilahti P, Jukema JW, Kahali B, Kahn RS, Kähönen

M, Kamstrup PR, Kanoni S, Kaprio J, Karaleftheri M, Kardia SL, Karpe F, Kee F, Keeman R, Kiemeney LA, Kitajima H, Kluivers KB, Kocher T, Komulainen P, Kontto J, Kooner JS, Kooperberg C, Kovacs P, Kriebel J, Kuivaniemi H, Küry S, Kuusisto J, La Bianca M, Laakso M, Lakka TA, Lange EM, Lange LA, Langefeld CD, Langenberg C, Larson EB, Lee IT, Lehtimäki T, Lewis CE, Li H, Li J, Li-Gao R, Lin H, Lin LA, Lin X, Lind L, Lindström J, Linneberg A, Liu Y, Liu Y, Lophatananon A, Luan J, Lubitz SA, Lyytikäinen LP, Mackey DA, Madden PA, Manning AK, Männistö S, Marenne G, Marten J, Martin NG, Mazul AL, Meidtner K, Metspalu A, Mitchell P, Mohlke KL, Mook-Kanamori DO, Morgan A, Morris AD, Morris AP, Müller-Nurasyid M, Munroe PB, Nalls MA, Nauck M, Nelson CP, Neville M, Nielsen SF, Nikus K, Njølstad PR, Nordestgaard BG, Ntalla I, O'Connel JR, Oksa H, Loohuis LM, Ophoff RA, Owen KR, Packard CJ, Padmanabhan S, Palmer CN, Pasterkamp G, Patel AP, Pattie A, Pedersen O, Peissig PL, Peloso GM, Pennell CE, Perola M, Perry JA, Perry JR, Person TN, Pirie A, Polasek O, Posthuma D, Raitakari OT, Rasheed A, Rauramaa R, Reilly DF, Reiner AP, Renström F, Ridker PM, Rioux JD, Robertson N, Robino A, Rolandsson O, Rudan I, Ruth KS, Saleheen D, Salomaa V, Samani NJ, Sandow K, Sapkota Y, Sattar N, Schmidt MK, Schreiner PJ, Schulze MB, Scott RA, Segura-Lepe MP, Shah S, Sim X, Sivapalaratnam S, Small KS, Smith AV, Smith JA, Southam L, Spector TD, Speliotes EK, Starr JM, Steinthorsdottir V, Stringham HM, Stumvoll M, Surendran P, 't Hart LM, Tansey KE, Tardif JC, Taylor KD, Teumer A, Thompson DJ, Thorsteinsdottir U, Thuesen BH, Tönjes A, Tromp G, Trompet S, Tsafantakis E, Tuomilehto J, Tybjaerg-Hansen A, Tyrer JP, Uher R, Uitterlinden AG, Ulivi S, van der Laan SW, Van Der Leij AR, van Duijn CM, van Schoor NM, van Setten J, Varbo A, Varga TV, Varma R, Edwards DR, Vermeulen SH, Vestergaard H, Vitart V, Vogt TF, Vozzi D, Walker M, Wang F, Wang CA, Wang S, Wang Y, Wareham NJ, Warren HR, Wessel J, Willems SM, Wilson JG, Witte DR, Woods MO, Wu Y, Yaghootkar H, Yao J, Yao P, Yerges-Armstrong LM, Young R, Zeggini E, Zhan X, Zhang W, Zhao JH, Zhao W, Zhao W, Zheng H, Zhou W, Rotter JI, Boehnke M, Kathiresan S, McCarthy MI, Willer CJ, Stefansson K, Borecki IB, Liu DJ, North KE, Heard-Costa NL, Pers TH, Lindgren CM, Oxvig C, Kutalik Z, Rivadeneira F, Loos RJ, Frayling TM, Hirschhorn JN, Deloukas P, Lettre GEPIC-InterAct ConsortiumCHD Exome+ ConsortiumExomeBP ConsortiumT2D-Genes ConsortiumGoT2D Genes ConsortiumGlobal Lipids Genetics ConsortiumReproGen ConsortiumMAGIC Investigators. Rare and low-frequency coding variants alter human adult height. *Nature*. 2017;**542**(7640):186–190.

6. 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.
7. de Bruin C, Dauber A. Insights from exome sequencing for endocrine disorders. *Nat Rev Endocrinol*. 2015;**11**(8):455–464.
8. Gkourogianni A, Andrew M, Tyzinski L, Crocker M, Douglas J, Dunbar N, Fairchild J, Funari MF, Heath KE, Jorge AA, Kurtzman T, LaFranchi S, Lalani S, Lebl J, Lin Y, Los E, Newbern D, Nowak C, Olson M, Popovic J, Pruhová Š, Elblova L, Quintos JB, Segerlund E, Sentchordi L, Shinawi M, Stattin EL, Swartz J, Angel AG, Cuéllar SD, Hosono H, Sanchez-Lara PA, Hwa V, Baron J, Nilsson O, Dauber A. Clinical characterization of patients with autosomal dominant short stature due to aggrecan mutations. *J Clin Endocrinol Metab*. 2017;**102**(2):460–469.
9. Nilsson O, Guo MH, Dunbar N, Popovic J, Flynn D, Jacobsen C, Lui JC, Hirschhorn JN, Baron J, Dauber A. Short stature, accelerated bone maturation, and early growth cessation due to heterozygous aggrecan mutations. *J Clin Endocrinol Metab*. 2014;**99**(8):E1510–E1518.
10. de Bruin C, Finlayson C, Funari MFA, Vasques GA, Lucheze Freire B, Lerario AM, Andrew M, Hwa V, Dauber A, Jorge AAL. Two patients with severe short stature due to a FBN1 Mutation (p.Ala1728Val) with a mild form of acromicric dysplasia. *Horm Res Paediatr*. 2016;**86**(5):342–348.
11. Hisado-Oliva A, Ruzafa-Martin A, Sentchordi L, Funari MFA, Bezanilla-López C, Alonso-Bernáldez M, Barraza-García J, Rodriguez-Zabala M, Lerario AM, Benito-Sanz S, Aza-Carmona M, Campos-Barros A, Jorge AAL, Heath KE. Mutations in C-natriuretic peptide (NPPC): a novel cause of autosomal dominant short stature [published online ahead of print June 29, 2017]. *Genet Med*.
12. Gao B, Guo J, She C, Shu A, Yang M, Tan Z, Yang X, Guo S, Feng G, He L. Mutations in IHH, encoding Indian hedgehog, cause brachydactyly type A-1. *Nat Genet*. 2001;**28**(4):386–388.
13. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; 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.
14. Jarvik GP, Browning BL. Consideration of cosegregation in the pathogenicity classification of genomic variants. *Am J Hum Genet*. 2016;**98**(6):1077–1081.
15. Strande NT, Riggs ER, Buchanan AH, Ceyhan-Birsoy O, DiStefano M, Dwight SS, Goldstein J, Ghosh R, Seifert BA, Sneddon TP, Wright MW, Milko LV, Cherry JM, Giovanni MA, Murray MF, O'Daniel JM, Ramos EM, Santani AB, Scott AF, Plon SE, Rehm HL, Martin CL, Berg JS. Evaluating the clinical validity of gene-disease associations: an evidence-based framework developed by the Clinical Genome Resource. *Am J Hum Genet*. 2017;**100**(6):895–906.
16. Bonafe L, Cormier-Daire V, Hall C, Lachman R, Mortier G, Mundlos S, Nishimura G, Sangiorgi L, Savarirayan R, Sillence D, Spranger J, Superti-Furga A, Warman M, Unger S. Nosology and classification of genetic skeletal disorders: 2015 revision. *Am J Med Genet A*. 2015;**167A**(12):2869–2892.
17. Marchini A, Ogata T, Rappold GA. A Track Record on SHOX: From Basic Research to Complex Models and Therapy. *Endocr Rev*. 2016;**37**(4):417–448.
18. 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.
19. Kavran JM, Ward MD, Oladosu OO, Mulepati S, Leahy DJ. All mammalian Hedgehog proteins interact with cell adhesion molecule, down-regulated by oncogenes (CDO) and brother of CDO (BOC) in a conserved manner. *J Biol Chem*. 2010;**285**(32):24584–24590.
20. Pereira J, Johnson WE, O'Brien SJ, Jarvis ED, Zhang G, Gilbert MT, Vasconcelos V, Antunes A. Evolutionary genomics and adaptive evolution of the Hedgehog gene family (Shh, Ihh and Dhh) in vertebrates. *PLoS One*. 2014;**9**(12):e74132.
21. Minina E, Wenzel HM, Kreschel C, Karp S, Gaffield W, McMahon AP, Vortkamp A. BMP and Ihh/PTHrP signaling interact to coordinate chondrocyte proliferation and differentiation. *Development*. 2001;**128**(22):4523–4534.
22. Hellemans J, Coucke PJ, Giedion A, De Paepe A, Kramer P, Beemer F, Mortier GR. 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.
23. Liu M, Wang X, Cai Z, Tang Z, Cao K, Liang B, Ren X, Liu JY, Wang QK. 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.
24. Byrnes AM, Racacho L, Grimsey A, Hudgins L, Kwan AC, Sangalli M, Kidd A, Yaron Y, Lau YL, Nikkel SM, Bulman DE. 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.

25. Wallis D, Muenke M. Mutations in holoprosencephaly. *Hum Mutat*. 2000;**16**(2):99–108.
26. Roessler E, El-Jaick KB, Dubourg C, Vélez JI, Solomon BD, Pineda-Alvarez DE, Lacbawan F, Zhou N, Ouspenskaia M, Paulussen A, Smeets HJ, Hehr U, Bendavid C, Bale S, Odent S, David V, Muenke M. The mutational spectrum of holoprosencephaly-associated changes within the SHH gene in humans predicts loss-of-function through either key structural alterations of the ligand or its altered synthesis. *Hum Mutat*. 2009;**30**(10):E921–E935.
27. Solomon BD, Bear KA, Wyllie A, Keaton AA, Dubourg C, David V, Mercier S, Odent S, Hehr U, Paulussen A, Clegg NJ, Delgado MR, Bale SJ, Lacbawan F, Ardinger HH, Aylsworth AS, Bhengu NL, Braddock S, Brookhyser K, Burton B, Gaspar H, Grix A, Horovitz D, Kanetzke E, Kayserili H, Lev D, Nikkel SM, Norton M, Roberts R, Saal H, Schaefer GB, Schneider A, Smith EK, Sowry E, Spence MA, Shalev SA, Steiner CE, Thompson EM, Winder TL, Balog JZ, Hadley DW, Zhou N, Pineda-Alvarez DE, Roessler E, Muenke M. Genotypic and phenotypic analysis of 396 individuals with mutations in Sonic Hedgehog. *J Med Genet*. 2012;**49**(7):473–479.
28. Williams KD, Nahhas RW, Cottom CR, Lawrence S, Subedi J, Jha B, Czerwinski SA, Blangero J, Williams-Blangero S, Towne B. Evaluation of qualitative methods for phenotyping brachymesophalangia-V from radiographs of children. *Am J Hum Biol*. 2012;**24**(1):68–73.
29. Finkelstein BS, Imperiale TF, Speroff T, Marrero U, Radcliffe DJ, Cuttler L. Effect of growth hormone therapy on height in children with idiopathic short stature: a meta-analysis. *Arch Pediatr Adolesc Med*. 2002;**156**(3):230–240.
30. Lee PA, Sävendahl L, Oliver I, Tauber M, Blankenstein O, Ross J, Snajderova M, Rakov V, Pedersen BT, Christesen HT. Comparison of response to 2-years' growth hormone treatment in children with isolated growth hormone deficiency, born small for gestational age, idiopathic short stature, or multiple pituitary hormone deficiency: combined results from two large observational studies. *Int J Pediatr Endocrinol*. 2012;**2012**(1):22.
31. Bakker B, Frane J, Anhalt H, Lippe B, Rosenfeld RG. Height velocity targets from the national cooperative growth study for first-year growth hormone responses in short children. *J Clin Endocrinol Metab*. 2008;**93**(2):352–357.