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FACULDADE DE MEDICINA

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Haploinsuficiência do *SHOX*: modificadores genéticos do fenótipo e resposta ao tratamento com hormônio de crescimento

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NAIARA CASTELO BRANCO DANTAS

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Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutora em Ciências

Programa de Endocrinologia

Orientador: Prof. Dr. Alexander Augusto de Lima Jorge

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“Somos assim: sonhamos o voo, mas tememos a altura.

Para voar é preciso ter coragem para enfrentar o terror do vazio.

Porque é só no vazio que o voo acontece.

O vazio é o espaço da liberdade, a ausência de certezas.

Mas isso é o que tememos: o não ter certezas.

Por isso, trocamos o voo por gaiolas.

As gaiolas são o lugar onde as certezas moram.”

Rubem Alves

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RESUMO

Dantas NCB. Haploinsuficiência do *SHOX*: modificadores genéticos do fenótipo e resposta ao tratamento com hormônio de crescimento [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2023.

Introdução: A haploinsuficiência do *SHOX* é a principal causa de baixa estatura monogênica. Alterações em heterozigose nesse gene são responsáveis por 2-10% dos casos de baixa estatura isolada e 70-90% dos casos de Discondrosteose de Léri-Weill. Além disso, alterações no gene *SHOX* contribuem para a baixa estatura e alterações esqueléticas encontradas na Síndrome de Turner (ST). O fenótipo associado a haploinsuficiência do *SHOX* é caracterizado por uma ampla variabilidade fenotípica entre os indivíduos. Outro ponto é que na literatura existem poucos estudos comparando a altura adulta de paciente tratados com hormônio de crescimento (GH) com um grupo não tratado tanto na haploinsuficiência do *SHOX* isolada como na ST. **Objetivos**: Avaliar o impacto de variantes genéticas na determinação do fenotípica em indivíduos com deficiência do *SHOX* e analisar a efetividade do tratamento com GH na melhora da altura adulta na haploinsuficiência do *SHOX* e na ST. **Métodos**: O estudo foi dividido em 3 partes: 1ª parte - para a avaliação de variantes genéticas foram selecionados 98 indivíduos de 48 famílias com haploinsuficiência do *SHOX* para estudo através de um painel customizado projetado para capturar toda a região genômica do *SHOX* e 114 outros genes que modulam o crescimento e/ou ação do *SHOX*. 2ª Parte - para análise da altura adulta na haploinsuficiência do *SHOX*, quarenta e sete pacientes foram incluídos em um estudo retrospectivo longitudinal onde um grupo não tratado foi comparado com um grupo tratado com GH. A altura adulta foi alcançada em 13 indivíduos não tratados e 18 tratados com GH. 3ª Parte - para análise da altura adulta na ST, foram incluídos 131 pacientes não tratados e 168 tratados com GH em um estudo retrospectivo. **Resultados**: 1ª parte - na análise das variantes genéticas, encontramos oito variantes em heterozigose em 11 indivíduos de nove famílias em genes com potencial papel de modificadores genéticos. Foram encontradas variante provavelmente patogênica nos genes *CYP26C1*, *PTHLH* e

ACAN, e variantes de significância incerta nos genes *NPR2*, *RUNX2* e *TP53* em indivíduos com o fenótipo mais grave das famílias com haploinsuficiência do *SHOX*. Famílias com alteração restrita à região regulatória do *SHOX* tiveram maior prevalência de uma segunda variante provável patogênica (27%) do que famílias com alteração comprometendo a região codificadora do *SHOX* (2,9%, $p = 0,04$). 2ª Parte - na avaliação da altura adulta nos indivíduos com haploinsuficiência do *SHOX*, o grupo não- tratado teve piora do desvio-padrão (DP) da altura durante o seguimento até atingir altura adulta (-0,8 [-1,1;−0,4]), com aumento na prevalência de baixa estatura de 31% para 77%. Por outro lado, o grupo tratado com GH teve uma melhora no DP da altura desde o início do tratamento até a altura adulta (0,6 [0,2; 0,6]; $p < 0,001$), atingindo altura adulta 1 DP (6,3 cm) maior que os não tratados. Quanto ao uso de análogos de hormônio liberador de gonadotrofina (aGnRH), os subgrupos (GH sozinho ou mais aGnRH) atingiram altura adulta semelhante, apesar da maior prevalência de paciente púberes e pior previsão de altura adulta no início do tratamento com GH no grupo que usou terapia combinada. 3ª Parte - nos pacientes com ST, no final do seguimento, o grupo tratado com GH era 6,2 cm mais alto que o grupo não tratado (altura adulta = 149 cm vs. 142,8 cm, $p < 0,001$). **Conclusão**: Variantes em genes relacionados à placa de crescimento têm um potencial papel como modificadores genéticos do fenótipo em indivíduos com haploinsuficiência do *SHOX*. Em indivíduos com alterações restritas à região regulatória, uma segunda alteração genética pode ser crítica para determinação da penetrância e expressão do fenótipo. O uso do tratamento com GH melhora altura adulta em pacientes com haploinsuficiência *SHOX* e ST. Em pacientes peripuberais com haploinsuficiência do *SHOX*, a adição de aGnRH permite a obtenção de altura adulta semelhante à altura adulta de pacientes que iniciam apenas GH na idade pré-púbere.

Palavras-chave: Proteína de homoeobox de baixa estatura. Estatura/genética. Crescimento/genética. Insuficiência de crescimento. Antropometria. Hormônio do crescimento/uso terapêutico. Variabilidade fenotípica. Genes modificadores. Genética humana. Sequenciamento de nucleotídeos em larga escala.

ABSTRACT

Dantas NCB. *SHOX* haploinsufficiency: genetic modifiers of the phenotype and response to growth hormone treatment [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2023.

Introduction: *SHOX* haploinsufficiency is the most common monogenic cause of short stature. Heterozygous defects in this gene are responsible for 2-10% of cases of isolated short stature and 70-90% of cases of Léri-Weill Dyschondrosteosis. Furthermore, *SHOX* gene alteration contributes to short stature and skeletal abnormalities of Turner syndrome (TS). The phenotype associated with *SHOX* haploinsufficiency is characterized by a wide phenotypic variability between individuals. Another point is that in the literature there are few studies comparing the adult height of patients treated with growth hormone (rhGH) with an untreated group in both isolated *SHOX* haploinsufficiency and TS. **Objectives**: To evaluate the impact of genetic variants on phenotypic determination in individuals with *SHOX* deficiency and to analyze the effectiveness of rhGH treatment in improving adult height in *SHOX* haploinsufficiency and TS. **Methods**: The study was divided into 3 parts: 1st part - to evaluate genetic variants with potential role as phenotypic modifiers, 98 individuals from 48 families with *SHOX* haploinsufficiency were selected for study through a customized panel designed to capture the entire *SHOX* genomic region and 114 other genes that modulate the growth and/or action of *SHOX* .2nd Part - for analysis of adult height in *SHOX* haploinsufficiency, an untreated group was compared with a rhGH treated group, forty-seven patients were included in a longitudinal retrospective study. Adult height was achieved in 13 untreated and 18 rhGH-treated individuals. 3rd Part - for analysis of adult height in TS, 131 untreated and 168 rhGH-treated individuals were included in a retrospective study. **Results**: 1st part - in the analysis of genetic variants, we found eight heterozygous variants in 11 individuals from nine families in genes with a potential role as genetic modifiers. A likely pathogenic variant was found in the *CYP26C1*, *PTHLH* and *ACAN* genes, and variants of uncertain significance in the *NPR2*, *RUNX2* and *TP53* genes in individuals with the most severe phenotype of

families with *SHOX* haploinsufficiency. Families with an alteration restricted to the *SHOX* regulatory region had a higher prevalence of a second likely pathogenic variant (27%) than families with an alteration compromising the *SHOX* coding region (2.9%, *p* = 0.04). 2nd Part - when assessing adult height in individuals with *SHOX* haploinsufficiency, the untreated group had a worsening in the height standard deviation (SD) during follow-up until reaching adult height (-0.8 [-1.1;−0.4]), with an increase in the prevalence of short stature from 31% to 77%. On the other hand, the group treated with rhGH had an improvement in the height SD from baseline to adult height (0.6 [0.2; 0.6]; p < 0.001), reaching adult height 1 SD (6, 3 cm) taller than untreated ones. Regarding the use of gonadotropin-releasing hormone analogues (GnRHa), the subgroups (rhGH alone or plus GnRHa) reached similar adult height, despite the higher prevalence of pubertal patients and worse prediction of adult height at the beginning of treatment with rhGH in the group that used combination therapy. 3rd Part - in patients with TS, at the end of follow-up, the rhGH-treated group was 6.2 cm taller than the untreated group (adult height = 149 cm vs. 142.8 cm, *p* < 0.001). **Conclusion**: Variants in genes related to the growth plate have a potential role as genetic modifiers of the phenotype in individuals with *SHOX* haploinsufficiency. In individuals with alterations restricted to the regulatory region, a second genetic alteration may be critical to determine the penetrance and expression of the phenotype. The use of rhGH treatment improves adult height in patients with *SHOX* haploinsufficiency and TS. In peripubertal patients with *SHOX* haploinsufficiency, the addition of GnRHa to rhGH allows the achievement of adult height similar to the adult height of patients starting rhGH alone at prepubertal age.

Keywords: Short stature homeobox protein. Body height/genetics. Growth/genetics. Failure to thrive. Anthropometry. Growth hormone/therapeutic use. Phenotypic variability. Genes modifier. Human genetics. High-throughput nucleotide sequencing.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

ACAN	gene aggrecan
ACMG	Colégio Americano de Genética Médica e Genômica (The American College of Medical Genetics and Genomics)
aGnRH	análogos de hormônio liberador de gonadotrofina
AMP	Associação de Patologia Molecular (The Association for Molecular Pathology)
AR	ácido retinóico
BEI	baixa estatura isolada
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CBAEM	Congresso Brasileiro de Endocrinologia e Metabologia
cm	centímetros
CNE	elementos do DNA não codificante altamente conservado evolutivamente
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CNVs	variações no número de cópias
CYP26C1	gene citocromo P450 família 26 subfamília C membro 1
DL	Displasia mesomélica de Langer
DLW	Discondrosteose de Léri Weill
DNA	ácido desoxirribonucléico
DP	desvio-padrão
ECR/ECS	sequência evolutivamente conservada
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
FGFR3	gene do receptor 3 do fator de crescimento de fibroblasto
FMUSP	Faculdade de Medicina da Universidade de São Paulo

GH	hormônio do crescimento
JAK	Janus Quinase
Kb	kilobases
LIM	Laboratório de Investigação Médica
LOVD	banco de dados aberto *Leiden Open Variation Database*
MAPK	proteínas quinases ativadas por mitógenos
Mb	megabases
MIM	banco de dados: Mendelian Inheritance in Man
MLPA	amplificação dependente da ligação de múltiplas sondas (the multiplex ligation-dependent probe amplification)
n	número
NPR2	gene receptor do peptídeo natriurético B
OAR	opt, aristaless and rax
p	valor-p ou probabilidade de significância
PAR1	região pseudoautossômica 1
pb	pares de base
PHOG	pseudoautosomal homeobox-containing osteogenic gene
PTHLH	gene hormônio semelhante ao hormônio paratireóide
RUNX2	gene runt related transcription factor 2
SELA	Laboratório de Sequenciamento em Larga Escala
SHOX	*short stature homeobox containing gene*
SIN3A	gene proteína com hélices emparelhadas anfipáticas
SNVs	variantes de nucleotídeo único
ST	Síndrome de Turner
STAT	transdutor de sinal/ativador da transcrição
TP53	gene supressor de tumor

UNICAMP	Universidade Estadual de Campinas
USP-RP	Universidade de São Paulo – Campus Ribeirão Preto
USP-SP	Universidade de São Paulo – Campus São Paulo
Xp	braço curto do cromosomo X
Xq	braço longo do cromossomo X
Yp	braço curto do cromosomo Y

LISTA DE SÍMBOLOS

μ	média
σ	desvio-padrão
%	porcentagem
-	menos
+	mais
<	menor
>	maior
≤	menor ou igual
≥	maior ou igual

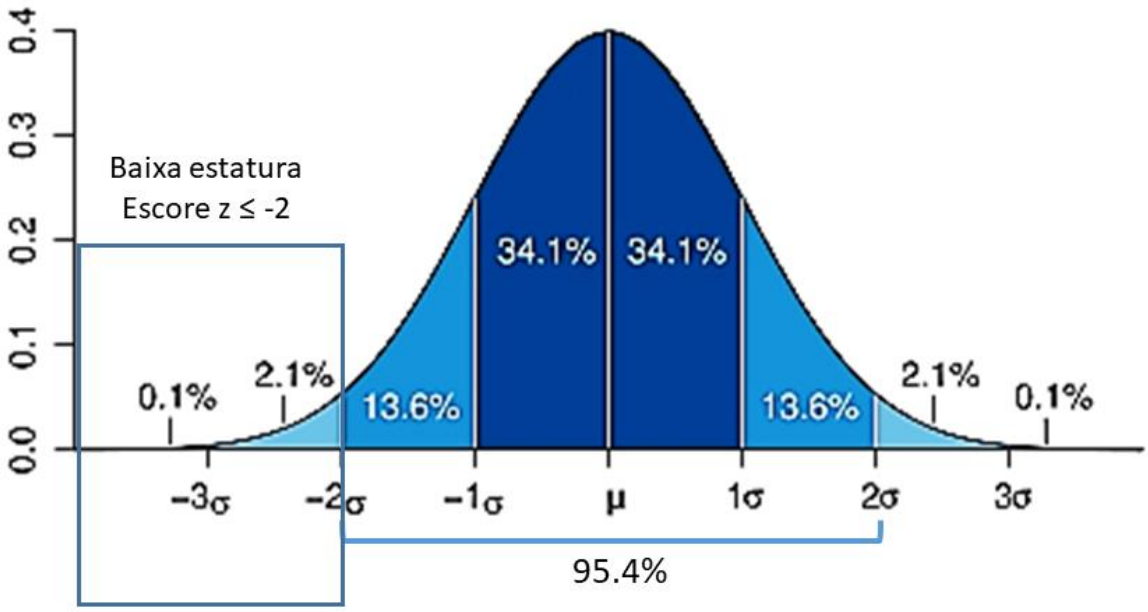
SUMÁRIO

1 INTRODUÇÃO

1 INTRODUÇÃO

Baixa estatura é uma queixa frequente nos consultórios de pediatria e endocrinologia, acometendo cerca de 2,1% da população pediátrica[1] (Figura 1). É estatisticamente definida como altura abaixo de dois desvios-padrão (DP) da média da altura ajustada para idade, sexo e grupo étnico (Escore-Z da altura ≤ -2) ou quando abaixo de dois DP da média da altura dos pais (Escore-Z da altura do paciente menos Escore-Z da altura alvo ≤ -2).

Figura 1 - Representação da curva de distribuição normal (Gaussiana) ilustrando os desvios-padrões



Legenda: A variável altura tem distribuição Gaussiana na população. μ: média; σ: desvio-padrão.

Fonte: Adaptado de Toews, 2023[2].

A altura final dos indivíduos é determinada pelo crescimento longitudinal dos ossos longos. Esse crescimento é influenciado por fatores genéticos e ambientais[1], porém os fatores ambientais são responsáveis por uma pequena parcela da variação

da altura. Desta forma, a altura é determinada principalmente por fatores genéticos, que podem corresponder a até 80% de sua variabilidade, indicando um alto grau de herdabilidade[3]. Alterações em mais de 150 genes já foram implicadas como causadoras de síndromes associadas a distúrbios do crescimento, porém a maioria dos casos de baixa estatura ainda permanece com causa desconhecida[4-6]. Entre as alterações já identificadas, a causa mais comum de baixa estatura monogênica são mutações no gene *short stature homeobox containing gene* (*SHOX*), [*Mendelian Inheritance in Man* (MIM) *312865][1].

1.1 HISTÓRIA DO GENE *SHOX*

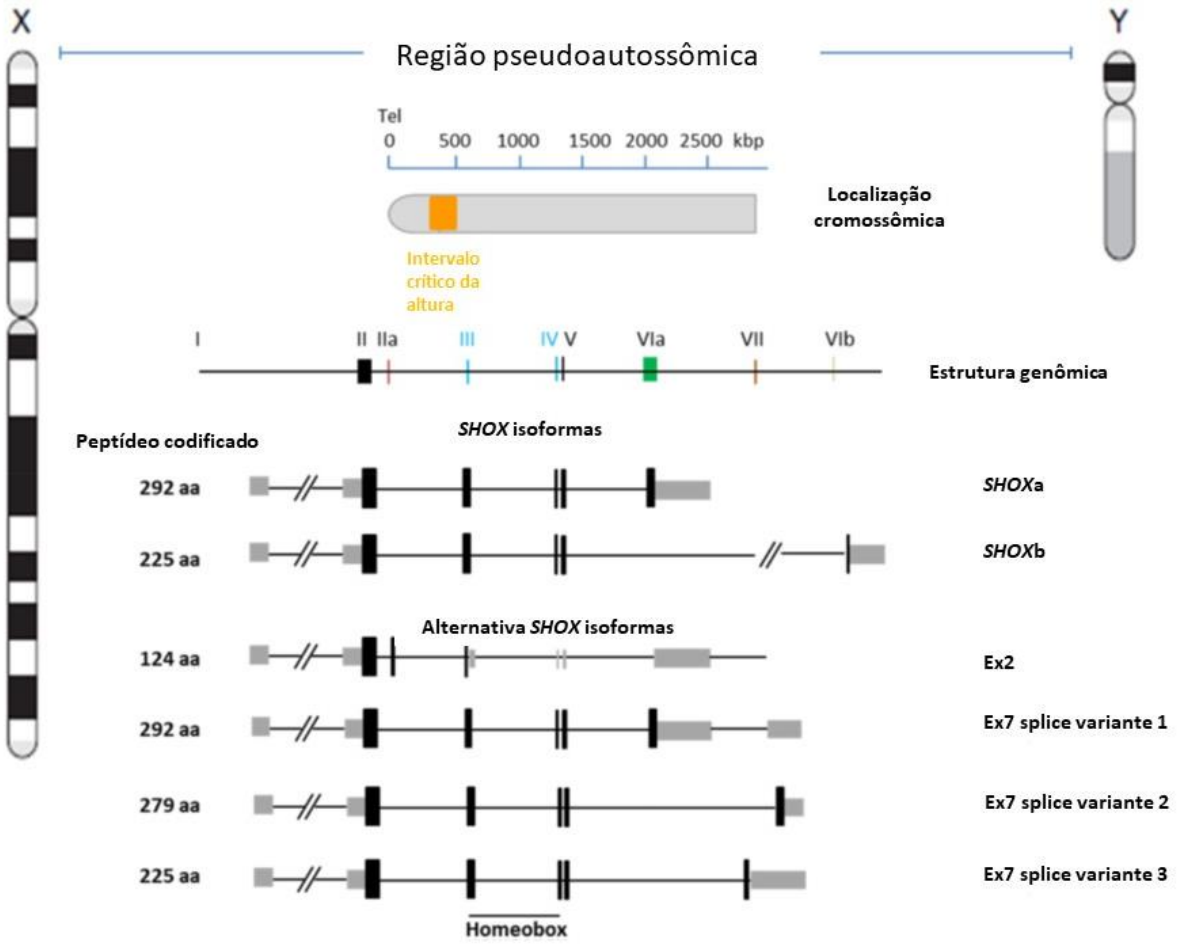
Em 1961, Jacobs e colaboradores[7] estudando um grupo de mulheres com amenorreia primária, perceberam que pacientes com deleções do braço curto do X (Xp) apresentavam baixa estatura enquanto aquelas que apresentavam apenas perda do braço longo do X (Xq) não tinham comprometimento da altura (Figura 2).

Analisando essa região Xp, Fisher e colaboradores[8] descreveram a presença de uma região homóloga entre os cromossomos X e Y que escapava da inativação do cromossomo X e que possivelmente estava associada à baixa estatura. Essa região é denominada de região pseudoautossômica 1 (PAR1), mede aproximadamente 4,6 megabases (Mb), e está localizada na porção distal do braço curto dos cromossomos sexuais (Xp e Yp)[9] (Figura 2). Está região é importante para o pareamento dos cromossomos homólogos X-Y durante a meiose masculina. Os genes abrigados dentro dessa região necessitam de duas cópias ativas para o funcionamento normal, e a perda de uma cópia desta região, haploinsuficiência, é associada à baixa estatura com padrão de herança pseudoautossômica dominante[8].

Dois grupos independentes estudando pacientes com baixa estatura e deleções envolvendo a PAR1, descreveram pela primeira vez um gene localizado nessa região associado ao fenótipo de baixa estatura. Rao e colaboradores[10] denominaram-no de *short stature homeobox containing gene* (*SHOX*), enquanto

Ellison e colaboradores[11] de *pseudoautosomal homeobox-containing osteogenic gene* (*PHOG*).

Figura 2 - Esquema representativo do gene *SHOX*



Legenda: O gene *SHOX* localiza-se na posição 505-527 kb distante do telômero dos cromossomos sexuais Xp22.33 e Yp11.32 e abrange aproximadamente 40kb. É composto por sete éxons que produzem dois transcritos principais, *SHOXa* e *SHOXb*. Os dois transcritos contêm uma sequência de ácido desoxirribonucléico (DNA) chamada *homeobox* que codifica o homeodomínio. As isoformas alternativas do *SHOX* também são formadas por *splicing* alternativo dos éxons.

Fonte: Adaptado de Marchini; Ogata; Rappold, 2016[1].

1.2 *SHORT STATURE HOMEOBOX CONTAINING GENE (SHOX)*

O gene *SHOX* está presente em algumas espécies de invertebrados e vertebrados, como chimpanzés, cães, rãs, frangos e peixes, exceto em roedores[1]. O gene possui 7 éxons (1 a 5, 6a e 6b), com aproximadamente 40 kilobases (Kb) de DNA genômico, distando 500 Kb do telômero. O éxon 1 possui 262 pares de bases (pb), não sendo traduzido, o éxon 2 possui 708 pb e sua porção 5' também não é traduzida, o éxon 3 possui 209 pb, o éxon 4 possui 58 pb, o éxon 5 possui 89 pb e o éxon 6a possui 1166 pb e o 6b possui 625 pb, ambos com grandes regiões não traduzidas[10,11].

Os éxons 3 e 4 contém uma sequência *homeobox* que codifica um domínio de 60 aminoácidos que é fundamental para que o SHOX se ligue a sequências específicas do DNA e atue como um fator ativador transcricional[10-12]. As proteínas que contém o homeodomínio se ligam a sequências palindrômicas do DNA 5' $TAAT(N)_nATTA$[13]. Para atuar como fator ativador transcricional, além do homeodomínio, o SHOX necessita de um outro domínio essencial para seu potencial de transativação, denominado *opt, aristaless and rax* (OAR), que possui 14 aminoácidos e é localizado na porção C-terminal[13].

Os éxons 6a e 6b possuem *splices* alternativos resultando em dois transcritos com padrão de expressão, distribuição tecidual, sítios de fosforilação e tamanhos diferentes, sendo eles, *SHOXa*, amplamente expresso em vários tecidos, e *SHOXb*, altamente expresso nos fibroblastos da medula óssea. O *SHOXb* não possui o domínio OAR, não sendo capaz de funcionar como um fator ativador transcricional[13]. O *SHOXa* e o *SHOXb* codificam proteínas de 292 e 225 aminoácidos, respectivamente. Outras isoformas codificadas são os éxons 2a, 7-1, 7-2 e 7-3, principalmente expressos na fase embrionária e provavelmente relacionados com a regulação da expressão espaciotemporal do *SHOX* (Figura 2)[14].

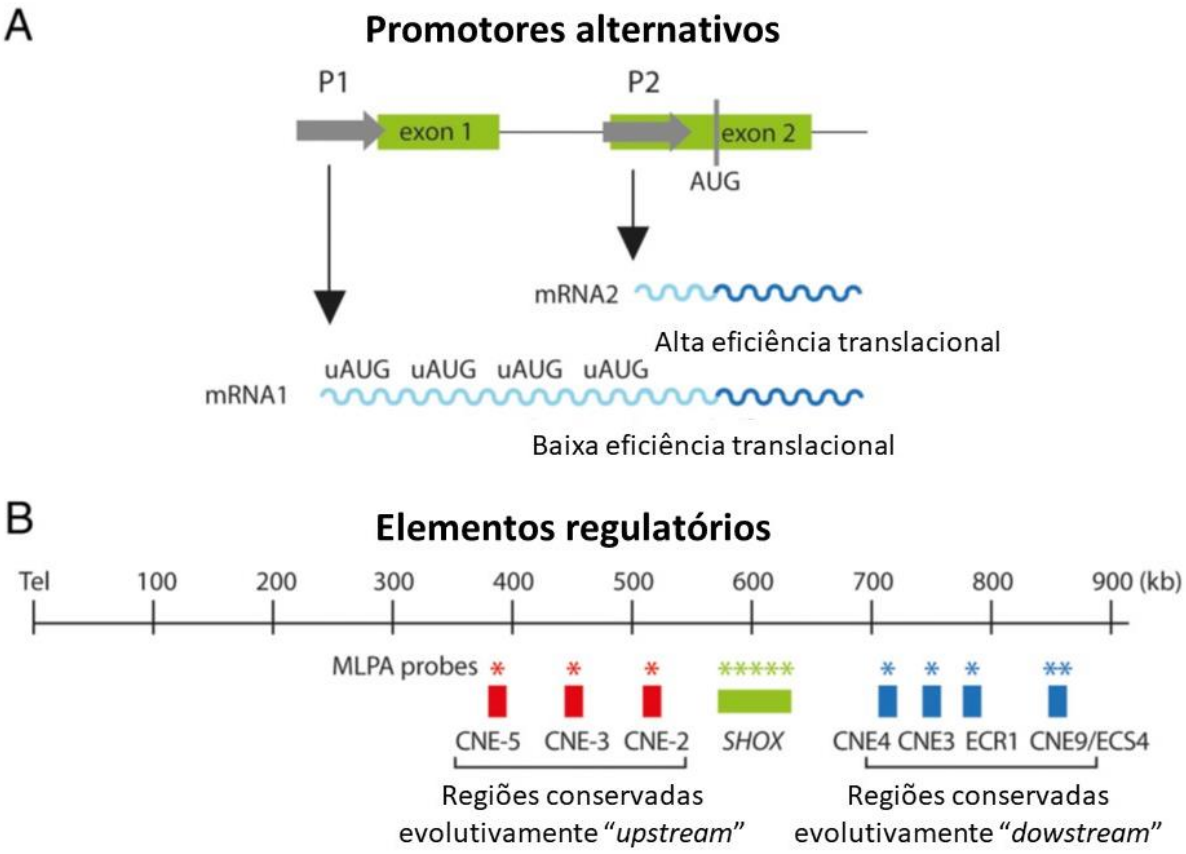
O gene *SHOX* se expressa em diferentes tecidos de acordo com a fase da vida e seu homeodomínio é importante na regulação da embriogênese e desenvolvimento[15]. Na fase embrionária, expressa-se nos membros e arcos

faríngeos, sendo encontrado a partir da 12ª semana nos condrócitos de embriões humanos[15]. Na fase pós-embrionária, é altamente expresso nas células osteogênicas e fibroblastos da medula óssea[12] e presente em menores proporções em músculos esqueléticos, coração, fígado e pâncreas[11,15].

O *SHOX* possuí regiões promotoras e regiões regulatórias que participam da regulação da expressão espaciotemporal do gene (Figura 3). As suas duas regiões promotoras são P1 e P2. P1 é localizada antes do éxon 1 não traduzido, e P2, na região não traduzida do éxon 2. Essas regiões promotoras codificam proteínas idênticas, porém P1 codifica um RNA mensageiro com 7 códons AUG a mais na porção 5' não traduzida que a região promotora P2, tendo menor eficiência translacional[16]. Os dois promotores podem ser usados em resposta a situações fisiológicas diferentes, mas o que determina qual região promotora será escolhida e os mecanismos moleculares que controlam a atividade das regiões promotoras não está claro[16].

Além das regiões promotoras, regiões regulatórias localizadas antes (*upstream)* e após (*downstream)* o gene *SHOX* (*SHOX area*) agem modulando a expressão do gene[17,18]. Esses elementos regulatórios atuam como sítios de ligação a fatores de transcrição e interagem com suas regiões promotoras modulando a expressão do gene. Até o momento, já foram descritas três regiões regulatórias *upstream* e quatro *downstream* ao gene[17,19-21] (Figura 3).

Figura 3 - Mecanismos regulatórios da expressão do *SHOX*



Legenda: A: Promotores alternativos. Dois promotores, P1 e P2, controlam a expressão do *SHOX*. B: Regiões evolutivamente conservadas na região pseudoautossômica 1. CNE: elementos do DNA não codificante altamente conservado evolutivamente. ECR/ECS: sequência evolutivamente conservada. A linha horizontal superior indica a distância física de Xp/ Yp telômero (Tel;hg19,build37). Posições genômicas: *SHOXa* (NM 000451.3), chrX:585.079 – 607 558; CNE-2, chrX:516, 610 –517 229; CNE-3, chrX:460.279 – 460 664; CNE-5, chrX:398,357–398 906; CNE4, chrX:714.085–714 740; CNE5, chrX:750.825–751 850; ECR1, xhrX:780,580 –781 235; e CNE9/ECS4, chrX:834.746 – 835 548 (Tel;hg19,build37).

Fonte: Adaptado de Marchini; Ogata; Rappold, 2016[1].

1.3 AÇÃO DO GENE *SHOX* NA CARTILAGEM DE CRESCIMENTO

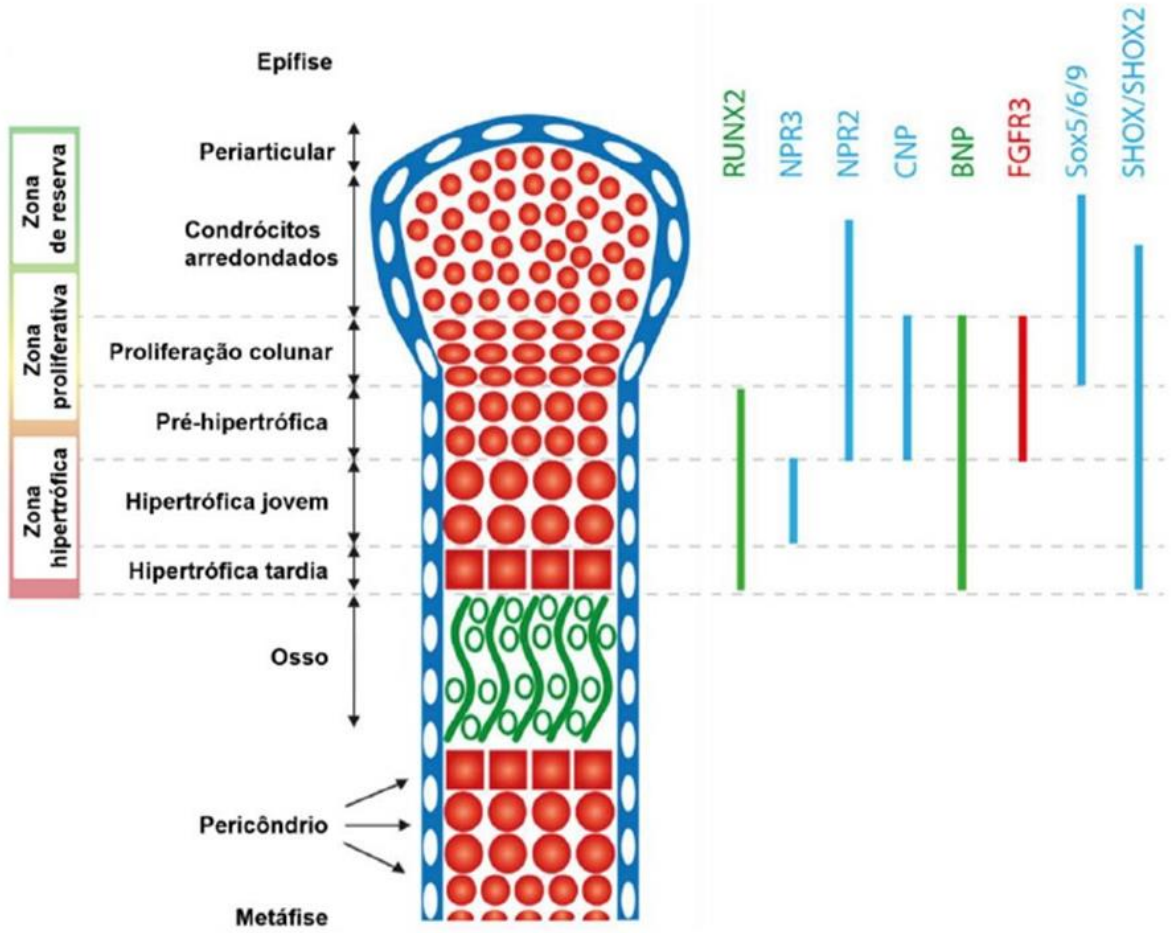
O crescimento longitudinal decorre da elongação que acontece na cartilagem de crescimento que é composta por 3 zonas, zona de repouso, proliferativa e hipertrófica[22]. A zona de repouso é composta por condrócitos não diferenciados, na

zona proliferativa esses condrócitos sofrem rápida proliferação gerando novos clones que ao pararem de proliferar formam a zona hipertrófica, onde os condrócitos aumentam o seu tamanho em 6 a 10 vezes (Figura 4)[22]. O gene *SHOX* é expresso principalmente na zona hipertrófica e em menor magnitude nas zonas de repouso e proliferativa, sugerindo seu envolvimento nas vias que regulam a proliferação e a maturação dos condrócitos (Figura 5)[15].

O *SHOX* interage direta ou indiretamente com genes importantes na regulação da cartilagem de crescimento como os gene do receptor 3 do fator de crescimento de fibroblasto (*FGFR3*, MIM *134934), gene receptor do peptídeo natriurético B (*NPR2*, MIM * 108961), gene runt related transcription factor 2 (*RUNX2,* MIM * 600211), entre outros (Figura 5)[1]. Exemplo dessa interação, é a sua interação com o *FGFR3*, que regula negativamente a proliferação e a diferenciação dos condrócitos, através da ativação das vias de sinalização Janus Quinase (JAK) e transdutor de sinal/ativador da transcrição (STAT) e proteínas quinases ativadas por mitógenos (MAPK)[23]. O *SHOX* inibe a transcrição do *FGFR3*, permitindo a proliferação e maturação normal dos condrócitos[23].

A importância da conservação da ação do gene *SHOX* no desenvolvimento e na arquitetura da cartilagem de crescimento é vista quando se analisa histopatologicamente a cartilagem de crescimento de indivíduos com alteração desse gene. Nesses indivíduos, se observar a presença de desorganização dos condrócitos na zona proliferativa com os condrócitos dispostos em aglomerados relativamente pequenos, sem o arranjo paralelo normalmente visto nesta zona[24]. Alterações no gene *SHOX* são relacionadas a distúrbios do crescimento e malformações esqueléticas, como deformidade de Madelung.

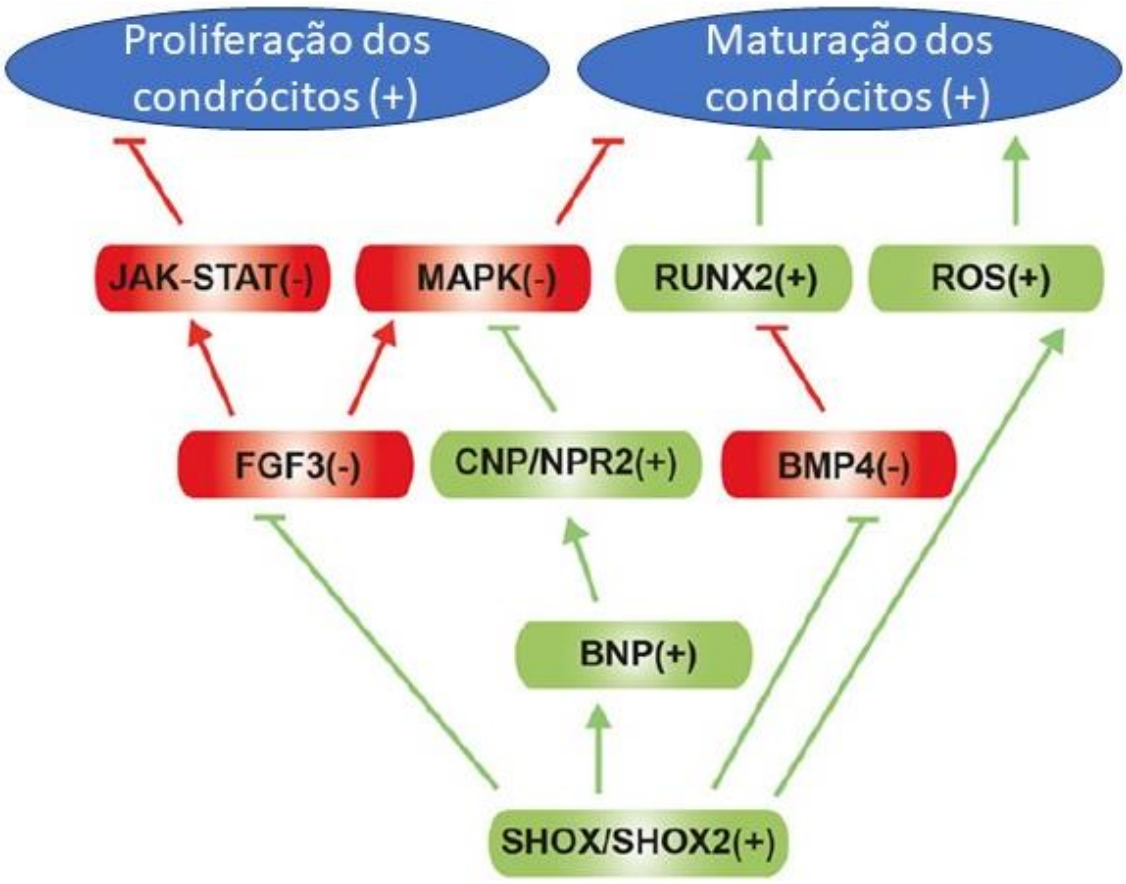
Figura 4 - Representação esquemática da cartilagem de crescimento



Legenda: A cartilagem de crescimento é dividida em 3 zonas que contém condrócitos em diferentes estágios de maturação, zona de repouso, proliferativa e hipertrófica. A direita da figura, representação do padrão de expressão na cartilagem de crescimento do gene *SHOX* e de fatores envolvidos na regulação da cartilagem de crescimentos que interagem com o *SHOX*.

Fonte: Adaptado de Marchini; Ogata; Rappold, 2016[1].

Figura 5 - Esquema ilustrando a interação do gene *SHOX* com genes relacionados a vias de regulação da proliferação e maturação dos condrócitos na cartilagem de crescimento



Legenda: Em verde as vias que estimulam e em vermelho as vias que inibem proliferação e maturação dos condrócitos.

Fonte: Adaptado de Marchini; Ogata; Rappold, 2016[1].

1.4 ALTERAÇÕES DO GENE *SHOX*

O *SHOX* se localiza em uma região com elevado conteúdo CG e alta incidência de sequências repetitivas, com alta taxa de recombinação durante a meiose, predispondo o gene a deleções ou duplicações[25,26]. Desta forma, 2/3 dos pacientes com defeitos no *SHOX* tem variações no número de cópias (CNVs), enquanto o

restante possui variantes de nucleotídeo único (SNVs) tipo *nonsense*, *missense* ou *frameshift*[27]. Alterações genéticas já descritas no gene *SHOX* podem ser acessadas através do banco de dados aberto *Leiden Open Variation Database* (LOVD) (https://databases.lovd.nl/shared/variants/SHOX/unique)[28].

Mutações heterozigotas no gene *SHOX* em regiões codificadoras ou regulatórias podem ser encontradas em 1-10% dos casos de baixa estatura isolada (BEI) (MIM:#300582)[10,29,30] e em 70-90% dos casos de discondrosteose de Léri Weill (DLW) (MIM:#127300)[31-33], caracterizada pela tríade de baixa estatura, Mesomelia e deformidade de Madelung. Mutações em homozigose nesse gene causam Displasia mesomélica de Langer (DL) (MIM: 249700), caracterizada por baixa estatura desproporcionada grave com encurtamento mesomélico[1,34,35]. Adicionalmente, a baixa estatura observada nos pacientes com Síndrome de Turner é parcialmente explicada pela perda de uma das cópias do gene *SHOX*[10].

Duplicações completas do *SHOX* já foram associadas ao fenótipo de alta estatura[36], como por exemplo na Síndrome de Klinefelter[37]. Enquanto, duplicações parciais envolvendo o gene e/ou suas regiões regulatórias foram associados ao fenótipo de baixa estatura e/ou DLW, porém a patogenicidade dessas duplicações parciais ainda não está bem estabelecida[38].

1.5 FENÓTIPOS DA HAPLOINSUFICIÊNCIA DO *SHOX*

Indivíduos com mutações em heterozigose do *SHOX* apresentam caracteristicamente baixa estatura desproporcional, com ou sem deformidade de Madelung e Mesomelia. A presença da desproporção corpórea, definida como escore-Z da relação da altura sentada pela altura total > 2, é observada em 88% das crianças e 96% dos adultos com haploinsuficiência do *SHOX*[39], sendo uma das características fenotípicas mais comuns nos portadores de defeitos neste gene[31].

A deformidade de Madelung é encontrada em 41% das crianças e 52% dos adultos com alterações em heterozigose do *SHOX*[39]. Provavelmente sua fisiopatologia decorrente do crescimento desorganizado da epífise radial, ocasionando curvatura e fusão prematura da epífise do rádio, luxação dorsal da ulna e ossos do carpo em cunha[40], sendo mais prevalente em mulheres, possivelmente devido a maior ação do estrogênio sobre a dinâmica óssea, de forma a acentuar a má-formação[34,41]. Radiograficamente, a presença da deformidade de Madelung é caracterizada pela translucência radial, subluxação da cabeça da ulna, encunhamento do carpo e alteração do ângulo do 3º metacarpo[41]. Além desses achados, os pacientes também podem apresentar palato ogival, cúbito valgo, escoliose, hipertrofia muscular, relação comprimento dos braços pela altura aumentada, micrognatia, anormalidades do pavilhão auricular, pescoço curto e encurvamento e encurtamento do antebraço[42].

Apesar dessas características serem bastante prevalentes em portadores de mutações do *SHOX*, corroborando com a alta penetrância que esta alteração genética apresenta, está condição não possuí uma correlação genótipo-fenótipo bem estabelecida assim como tem grande variabilidade fenotípica quanto a gravidade da baixa estatura, da desproporção corporal e do desenvolvimento da deformidade de Madelung, mesmo entre indivíduos de uma mesma família[31,41].

1.6 CORRELAÇÃO GENÓTIPO - FENÓTIPO

Até o momento, não foi identificada uma correlação genótipo-fenótipo clara nas alterações envolvendo o gene *SHOX*. No entanto, deleções envolvendo as regiões regulatórias *downstream* ao *SHOX* são associadas a um fenótipo menos grave e menor prevalência de sinais dismórficos (cúbito valgo, encurtamento dos membros superiores e inferiores) quando comparadas a alterações envolvendo a região codificadora do gene[34,43]. No estudo de Rosilio e colaboradores[34], deleções envolvendo a região *downstream* foram mais prevalentes na população com BEI (59%) do que na população com DLW (69%). Nesse mesmo estudo, a altura dos pais afetados com deleções isoladas da região regulatória *downstream* era menos

comprometida do que dos pais com alterações envolvendo a região codificadora do gene[34].

Além disso, deleções restritas as regiões regulatórias são associadas a reduzida penetrância[20]. Benito-Sanz e colaboradores[20], ao descrever a deleção recorrente da PAR1 de 47 Kb *downstream* ao *SHOX* (correspondente a região ECR1 representada na figura 3), identificou dentro das famílias, indivíduos portadores da deleção de 47 Kb que apresentavam o fenótipo de BEI, DLW ou indivíduos sem fenótipo característico.

1.7 VARIABILIDADE FENOTÍPICA NA HAPLOINSUFICIÊNCIA DO *SHOX*

Indivíduos com defeitos no gene *SHOX* apresentam grande variabilidade fenotípica. Fatores genéticos, chamados de modificadores genéticos, podem ser implicados como parte responsável por essa variabilidade[44]. Modificadores genéticos são alterações presentes em um segundo locus, capazes de modificar a gravidade do fenótipo quando a alteração genética primária é necessária e suficiente para causar a doença. Por outro lado, quando a presença de um segundo locus é determinante para manifestação da doença, temos a caracterização de uma herança digênica ou oligogênica[44]. Os modificadores genéticos podem ter diferentes tipos de ações, tais como: 1) alteração da penetrância - proporção de indivíduos carreando o locus primário que são fenotipicamente afetados; 2) modificação de dominância - situação onde em um determinado contexto genético (*genetic background*), a alteração em heterozigose é suficiente para induzir o fenótipo, e em outro determinado contexto genético, são necessárias alterações bialélicas para induzir o fenótipo; 3) expressividade - em indivíduos afetados, a presença do modificador genético atenua ou exacerba o fenótipo e 4) pleiotropia - a presença do modificador genético é capaz de alterar a combinações de fenótipos que os indivíduos portadores da alteração no locus primário apresentam[45,46].

Acredita-se que existam fatores genéticos nas regiões promotoras ou reguladoras próximas ao gene ou mesmo em outros genes capazes de modular o fenótipo em portadores da haploinsuficiência do *SHOX*, e que a identificação desses fatores possa melhor predizer o fenótipo dos indivíduos com esta condição. A análise dos modificadores genéticos do fenotípo da haploinsuficiência do *SHOX* foi objeto de estudo do artigo no capítulo 1[47], em resultados.

1.8 ALTURA ADULTA NOS INDIVÍDUOS COM ALTERAÇÃO DO *SHOX*

Indivíduos com haploinsuficiência do gene *SHOX* tem altura adulta 20 centímetros (cm) menor que a população feminina geral na Síndrome de Turner (ST)[48] e em média atingem altura adulta com desvio-padrão de -2,5 nos indivíduos com BEI ou DLW[32].

Apesar da maioria dos pacientes com alterações relacionadas ao gene *SHOX* não serem portadores de deficiência do hormônio do crescimento (GH), o tratamento com GH melhora a altura final destes indivíduos, com ganho estimado na altura final nos indivíduos tratados de 5-8 cm nos portadores de ST49 e 0,8 DP na BEI ou DLW[50]. No entanto, a maioria dos estudos não tem um grupo controle não tratado, baseando o ganho de altura final no ganho em relação à altura adulta predita no início do estudo ou a uma coorte histórica[50-55]. A proposta do estudo foi investigar o ganho de altura adulta decorrente do tratamento com GH comparando um grupo tratado com um grupo não tratado.

A análise da altura adulta de pacientes com haploinsuficiência do *SHOX* tratados com GH (n = 18) comparada com a altura adulta de não tratados (n = 13) foi objeto de estudo do artigo no capítulo 2[56], em resultados.

A análise da altura adulta de uma grande coorte de pacientes com Síndrome de Turner tratados com GH (n = 168) comparada com a altura adulta de não tratados (n = 131) foi objeto de estudo do artigo no capítulo 3[57], em resultados.

2 OBJETIVOS

2 OBJETIVOS

2.1 OBJETIVO GERAL

Essa tese tem como objetivo analisar uma casuística de indivíduos com alterações do gene *SHOX* com o objetivo de compreender os fenótipos associados a essa alteração com foco na variabilidade fenotípica e altura adulta.

2.2 OBJETIVOS ESPECÍFICOS

a) Identificar fatores genéticos capazes de atuar como moduladores do fenótipo em indivíduos com haploinsuficiência do gene *SHOX*;

b) Analisar a altura adulta dos indivíduos com haploinsuficiência do gene *SHOX* avaliando a efetividade do tratamento com hormônio de crescimento;

c) Analisar a altura adulta dos indivíduos com Síndrome de Turner avaliando a efetividade do tratamento com hormônio de crescimento.

3 RESULTADOS

3 RESULTADOS

3.1 PRÓLOGO

Os resultados dos estudos propostos são apresentados em três capítulos, cada um corresponde a um artigo científico que atende a um objetivo específico. Esses estudos foram revisados por pares e publicados em revistas especializadas e fazem parte da literatura científica dos estudos em baixa estatura. Os resultados do trabalho serão expostos na forma que foram preparados para publicação. Os artigos contaram com a contribuição fundamental de outros pesquisadores para sua elaboração, os quais estão destacados na autoria dos manuscritos. A descrição específica da metodologia utilizada e dos resultados será revelada no corpo do texto apresentado.

Além desses três artigos de pesquisa, outras contribuições científicas são apresentadas no anexo (Anexo A) deste documento. Estes constituem principalmente estudos liderados por outros colegas, onde tive a oportunidade de contribuir. No seu conjunto, este documento resume todos os trabalhos científicos desenvolvidos ao longo do curso de doutoramento.

3.2 CAPÍTULO 1: IDENTIFICATION OF A SECOND GENETIC ALTERATION IN PATIENTS WITH *SHOX* DEFICIENCY INDIVIDUALS: A POTENTIAL EXPLANATION FOR PHENOTYPE VARIABILITY

Dantas NCB, Funari MFA, Lerário AM, Andrade NLM, Rezende RC, Cellin LP, Alves C, Crisostomo LG, Arnhold IJP, Mendonca B, Scalco RC, Jorge AAL. Identification of a second genetic alteration in patients with SHOX deficiency individuals: a potential explanation for phenotype variability. Eur J Endocrinol. 2023;189(3):387-395. doi: 10.1093/ejendo/lvad128.

No nosso serviço, acompanhamos uma grande coorte de indivíduos com alteração envolvendo o gene *SHOX*. Nesses indivíduos, observamos uma ampla variabilidade fenotípica mesmo dentro da mesma família com a mesma alteração gênica, fato já descrito na literatura[31,41]. Nos últimos anos, o conhecimento sobre a existência de fatores modificadores do fenótipo em diversas doenças cresceu vertiginosamente[44]. Em 2016, Montalbano e colaboradores[58], descreveram alterações no gene *CYP26C1* capazes de modular o fenótipo em indivíduos com haploinsuficiência do *SHOX*. Deste então, não houve novos estudos procurando outros fatores genéticos capazes de modular o fenótipo na haploinsuficiência do *SHOX*. Desta forma, neste estudo, nos propomos a entender melhor os fatores genéticos capazes de atuar como modificadores do fenótipo na haploinsuficiência do *SHOX* que pudessem explicar a variabilidade fenotípica entre os indivíduos através de um painel customizado.

Os resultados desse estudo foram apresentados no 35º Congresso Brasileiro de Endocrinologia e Metabologia (CBAEM) em setembro de 2022, na modalidade apresentação oral, sendo selecionado para concorrer a prêmio e ganhando o prêmio de 3º melhor trabalho na categoria de pesquisa translacional (Anexo B).

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Original Research

Identification of a second genetic alteration in patients with *SHOX* deficiency individuals: a potential explanation for phenotype variability

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Abstract

Objective: Our study aimed to assess the impact of genetic modifiers on the significant variation in phenotype that is observed in individuals with *SHOX* deficiency, which is the most prevalent monogenic cause of short stature.

Design and methods: We performed a genetic analysis in 98 individuals from 48 families with *SHOX* deficiency with a target panel designed to capture the entire *SHOX* genomic region and 114 other genes that modulate growth and/or SHOX action. We prioritized rare potentially deleterious variants.

Results: We did not identify potential deleterious variants in the promoter or intronic regions of the *SHOX* genomic *locus*. In contrast, we found eight heterozygous variants in 11 individuals from nine families in genes with a potential role as genetic modifiers. In addition to a previously described likely pathogenic (LP) variant in *CYP26C1* observed in two families, we identified LP variants in *PTHLH* and *ACAN*, and variants of uncertain significance in *NPR2*, *RUNX2*, and *TP53* in more affected individuals from families with *SHOX* deficiency. Families with a *SHOX* alteration restricted to the regulatory region had a higher prevalence of a second likely pathogenic variant (27%) than families with an alteration compromising the *SHOX* coding region (2.9%, $P = .04$).

Conclusion: In conclusion, variants in genes related to the growth plate have a potential role as genetic modifiers of the phenotype in individuals with *SHOX* deficiency. In individuals with *SHOX* alterations restricted to the regulatory region, a second alteration could be critical to determine the penetrance and expression of the phenotype.

Keywords: *SHOX* deficiency, genetic modifiers, phenotype variability, short stature, genetic background

Significance statement

The *SHOX* deficiency is the most common monogenic cause of short stature and has a marked phenotype variability. In this study, we find genetic variants in growth plate-related genes which can have a potential role as genetic modifiers of the phenotype. This finding was more prevalent in families with deletions restricted to the *SHOX* regulatory region than in families with an alteration in *SHOX* coding regions. The presence of a second variant in growth plate-related genes intensifies the dysregulation of chondrocytes in the growth plate zones related to a primary alteration in the *SHOX* gene lead to a more severe phenotype. Our study shed light on the issue of co-occurrence of second genetic alteration in patients with *SHOX* deficiency.

Introduction

The short stature homeobox-containing gene (*SHOX*) [Mendelian Inheritance in Man (MIM)*312865] is harbored in the pseudoautosomal region 1, which is located in the distal part of the short arm of the X and Y chromosomes.[1,2] It escapes from X inactivation, thereby preserving two functional copies, both of which are necessary for adequate endochondral ossification and, consequently, for normal longitudinal

N.C.B.D. and M.F.A.F. contributed equally.

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growth.[2] SHOX protein is mainly expressed in hypertrophic chondrocytes, acting as a transcriptional factor regulating cell growth arrest and apoptosis. Loss of one copy of this gene reduces the amount of protein produced.[3]

SHOX deficiency is the most common monogenic cause of short stature.[4] Heterozygous alterations are responsible for 2%-10% of cases of idiopathic short stature (ISS) (MIM#300582)[2,5,6] and 70%-90% of individuals with Léri-Weill dyschondrosteosis (LWD) (MIM#127300),[7–9] a condition characterized by the triad of short stature, mesomelia, and Madelung's deformity.[10] The *SHOX* deficiency phenotype is widely variable, ranging from a normal height without any discernible alteration to severe short stature with or without body disproportion and/or Madelung's deformity. This variability can even occur within the same family, in individuals carrying the same alteration.[7,11] To better understand this phenotypic variability, Montalbano *et al.*[12] studied a large family in which some individuals displayed the LWD phenotype while others carrying the same *SHOX* missense mutation were phenotypically unaffected. Analysis of individuals by whole exome sequencing (WES) identified a *CYP26C1* (cytochrome P450 Family 26 Subfamily C Member 1, MIM: *608428) variant segregating with the LWD phenotype.[12] The CYP26C1 enzyme acts by catabolizing retinoic acid (RA) and regulating its level in cells. Retinoic acid is a potent regulator of cell proliferation and has a negative effect on *SHOX* expression.[12] *CYP26C1* defects result in increased levels of RA. The consequent decrease in *SHOX* expression leads to a more severe phenotype.[12]

In individuals with *SHOX* deficiency, variations in the expression of the remaining *SHOX* functional allele can result in differences in phenotype.[4] Additionally, variants in other genes affecting steps in the same biochemical pathway can alter *SHOX* function directly or indirectly by exerting an additive effect that modulates the final phenotype.[4] Therefore, the final *SHOX* phenotype results from the interaction of complex and variable factors affecting gene expression in each individual.

The aim of this investigation was to evaluate the influence of genetic variants on the phenotype of *SHOX* deficiency that could explain phenotype variability. We analyzed 98 individuals with *SHOX* deficiency with a custom target panel composed of genes from the *SHOX* gene network or the RA pathway, and genes related to the growth plate and short stature. We report the identification of second variants with a potential role as genetic modifiers, mostly present in families with a deletion restricted to the *SHOX* regulatory region.

Materials and methods

Subjects

This study was approved by the Research Ethics Committee of the University of Sao Paulo Medical School General Hospital and was conducted in accordance with the principles of the Declaration of Helsinki. Parental consent for molecular analysis and clinical information assessment was obtained after full explanation of the purpose of this study.

We included 123 individuals with *SHOX* deficiency followed-up in our out-patient clinic (Figure 1). Their *SHOX* deficiency diagnosis was obtained by multiplex ligation-dependent probe amplification (MLPA) analysis and Sanger sequencing, as previously described[9,10] (Figure S1). To search for variants that could act as genetic modifiers of *SHOX* deficiency, we selected for analysis with a custom panel 98 individuals from the initial cohort who represented extremes of the phenotype in each family and/or individuals with *SHOX* defects restricted to the regulatory region.

The individuals selected for presenting extremes of phenotype in each family (80 from 34 families) had alterations in the coding region of *SHOX* and differed from their relatives in their severity of short stature, degree of body disproportion, and presence or absence of Madelung's deformity. These individuals were analyzed to identify discordant genetic variants that could explain their phenotypic variability despite having the same *SHOX* defect.

The individuals with *SHOX* alterations restricted to the regulatory region (18 from 11 families) had a less severe phenotype and a lower prevalence of dysmorphic signs.[13] They were all selected for analysis with our custom target panel to better understand the phenotype and penetrance in these regions.

Study protocol

All data were systematically collected from medical records. Individuals were phenotypically characterized by gestational age, birth weight, birth length, weight, height, sitting height, arm span, body mass index, presence or absence of Madelung's deformity, Rappold criteria (Table S1),[14] and Binder criteria.[15] The measurements were converted to standard deviations scores (SDS) using age and gender-specific norms.[16–18] Madelung's deformity was analyzed clinically and radiologically.

Molecular genetic analysis

Ninety-eight genomic DNA samples were analyzed by a custom panel of targeted sequencing based on the SureSelect^{XT} capture system (Agilent Technologies, Santa Clara, CA). We developed the custom target panel containing genes from the *SHOX* gene network (52 genes), genes in the RA pathway (18 genes), genes related to growth plate and short stature (44 genes), and the entire *SHOX* genomic region (NM_000451.3, chrX:585,079-607,558; GRCh37), including the 5′ and 3′ regulatory regions (Table S2). The selected genes were chosen based on reports in PubMed® and the STRING database.[19] Sequencing was performed in paired-end mode using the NextSeq 500 platform with NextSeq Mid output 2 × 150 (Illumina, San Diego, CA). Coverage of the all gene coding regions was over 98% at a 20× depth threshold. In-house bioinformatic analysis was performed as previously reported.[20] The sequences were aligned with the human reference assembly (UCSC GRCh37).

Variant assessment

The target panel sequencing data were filtered for rare variants [minor allele frequency (MAF) < .01] in public databases: Online Archive of Brazilian Mutations; ABraOM;[21] Genome Aggregation Database; gnomAD;[22] and SELAdb,[23] variants located in exonic regions and consensus splice site sequences. Subsequently, we filtered the variants according to their pathogenic potential, including loss-of-function variants and missense variants with prediction of pathogenicity in the REVEL *in silico* tool.[24] We prioritized variants that were discordant among members of the same family with different phenotypes and variants that segregated with a more severe phenotype. In addition, we analyzed variants in the promoter and intronic regions of the *SHOX* gene. We filtered the rare

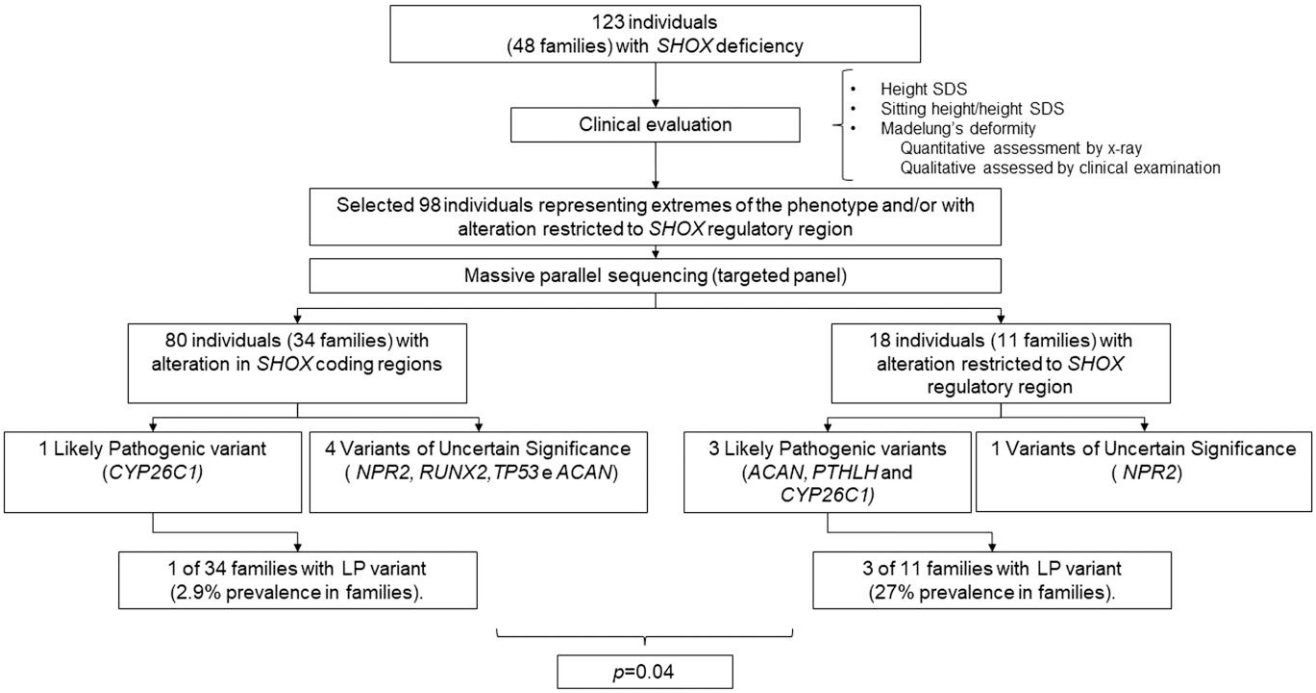

Figure 1. Flowchart of the cohort selection and summary of results. Fisher exact test was used to compare the group with alterations involving the *SHOX* coding region and the group with alterations restricted to the regulatory region. A *P*-value <.05 was used for statistical significance. LP, likely pathogenic.

variants and analyzed them in relation to the presence of transcription factors binding sites associated with *SHOX* expression using the USCS Genome Browser program (https://genome.ucsc.edu/).

The analysis of copy number variants (CNV) was performed using the CONTRA Software.[25] The variants were visually confirmed using the Integrative Genomics Viewer. The identified candidate variants were validated and segregated by Sanger sequencing. The identified CNV was confirmed by real-time PCR using a TaqMan™ Copy Number Assay (Applied Biosystems, Waltham, MA) to the parathyroid hormone-like hormone (*PTHLH*) (MIM*168470) (Hs03005427_cn) according to the manufacturer's protocol. Reactions were performed in QuantStudio™ 3 Real-time PCR System (Applied Biosystems, Waltham, MA), and data were analyzed with CopyCaller Software v2.1 (Thermo Fisher Scientific Inc., Waltham, MA) (Figure S2). The variants were classified in accordance with the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines (ACMG/AMP).[26]

Statistical analysis

Quantitative variables were expressed as median and interquartile range (p25-p75). Qualitative variables were expressed as percentages. Fisher exact test was used to compare the group. A *P*-value <.05 was used for statistical significance. All analyses were done with SigmaStat software (Systat Software, version 3.5, San Jose, CA).

Results

Clinical characteristics of patients with *SHOX* deficiency

Clinical characteristics of the 123 individuals with *SHOX* deficiency are depicted in Table 1. The cohort was predominant female (87%) with a high prevalence of short stature (70%) and body disproportion (85%). We compared individuals with alterations involving the coding region (83% of the cohort) with individuals with deletions in the regulatory region (17% of the cohort). There was a lower prevalence of Léri-Weill dyschondrosteosis and body disproportion in the latter group ($P = .015$ and $P < .001$, respectively).

Panel analysis

We analyzed 98 individuals with *SHOX* deficiency from 48 families by massive parallel sequencing (targeted panel) (Figure 1). We did not identify rare or potential deleterious variants in the promoter or intronic regions of the *SHOX* genomic region in our cohort. In contrast, we found 8 heterozygous variants in 11 individuals from nine families in genes with a potential role as genetic modifiers. Three variants were classified as likely pathogenic (LP) and five as variants of uncertain significance (VUS) (Table 2).

Potential genetic modifiers identified in individuals with an alteration encompassing the *SHOX* coding region

Five potential deleterious variants were identified in the most severely affected cases in families with a deletion or point mutation in the *SHOX* coding region, when affected family members differed in relation to severity of short stature and/or body disproportion and/or the presence of Madelung's deformity (Figure 2A, Table 2). In family 2, the proband's mother (I.1) had a more severe short stature (height SDS −4.2) and body disproportion (SH/H SDS 6.3), and presence of Madelung's deformity when compared with the proband (II.1, height SDS −1.1). The mother, in addition to a *SHOX* deletion involving all coding and regulatory regions, had a heterozygous VUS in the Natriuretic peptide receptor (*NPR2*)

Table 1. Clinical characteristics of subjects with *SHOX* deficiency.

	All patients with *SHOX* deficiency	*SHOX* genotypes		*P*-value[a]
		Gene disruptive mutation	CNVs involving regulatory regions	
Number of patients (n)	123	102	21	—
Gender (female: male)	71:52	57:45	14:7	.504
SHOX defect (%)				
• LoF mutation	56.9% (70/123)			
• Missense mutation	25.2%(31/123)			
• Deletion only in regulatory region	17.1%(21/123)			
• Duplication	.8%(1/123)			
ISS or NL: LWD phenotype (n)	67:56	50:52	17:4	.015
Gestational age (weeks)	39.0 (38.0-39.1) (n = 52)	39.0 (38.0-39.1) (n = 46)	38.5 (37.0-39.6) (n = 6)	.754
Birth weight SDS	−0.3 (−1.2-0.4) (n = 52)	−0.3 (−1.2-0.4) (n = 45)	−0.3 (−1.4 – −0.2) (n = 7)	.486
Birth length SDS	−1.4 (−2.5 – −0.9) (n = 48)	−1.4 (−2.5 – −0.6) (n = 41)	−1.8 (−2.1 – −1.3) (n = 7)	.704
Born SGA (%) (W, W and L, L)	(2%, 0%, 35.4%)	(2.2%, 0%, 36.6%)	(0%, 0%, 28.6%)	1.00;.0;1.00
First assessment in childhood[b]	48.8%	49.0%	38.1%	.501
Age at the first clinical evaluation (year)	12.3 (10.1-19.8) (n = 78)[c]	12.3 (10.4-19.9) (n = 68)	12.1 (7.1-15.4) (n = 10)	.450
Height SDS	−2.3 (−2.8-−1.6) (n = 121)	−2.4 (−2.8-−1.7) (n = 102)	−2.1 (−2.9-−1.3) (n = 19)	.190
Short stature (height SDS < −2.0) (%)	70%	69.6%	52.6%	.239
BMI				
<18 years old (BMI SDS)	0.6 (−0.1-1.5) (n = 56)	0.6 (−0.1-1.5) (n = 48)	1.0 (−0.3-1.3) (n = 8)	0.991
>18 years old (kg/m^2)	25.3 (22.8-31.3) (n = 36)	25.4 (22.8-31.2) (n = 29)	23.4 (22.6-31.4) (n = 7)	.873
SH/H SDS	3.4 (2.4-4.5) n = 106	3.6 (2.9-4.8) (n = 88)	1.6 (.7-2.7) (n = 18)	<.001
Arm span/height	0.970 (0.942-0.991) (n = 95)	0.968 (0.941-0.986) (n = 81)	0.994 (0.953-1.007) (n = 14)	0.051
Presence of Madelung's deformity (%)	42% (n = 119)	46.5% (n = 99)	20.0% (n = 20)	.053
Prevalence of body disproportion[d] (%)	84.9% (n = 106)	92.0% (n = 88)	50% (n = 18)	<.001
Binder criteria[e]	88.4% (n = 95)	91.4 (n = 81)	71.4 (n = 14)	.054
Rappold criteria				
• >4	63.8% (n = 94)	66.7% (n = 81)	46.1% (n = 13)	.214
• >7	47.9% (n = 94)	49.4% (n = 81)	38.5% (n = 13)	.665

Data are shown as median and interquartile range (25th-75th) or frequencies. [a]Difference between the gene disruptive mutation group and the CNVs involving regulatory regions group. [b]Frequency of individuals assessed for the first time before 18 years old. [c]Forty-three individuals evaluated in adulthood did not have chronological age data. [d]Body disproportion: defined as sitting height/height (SH/H) > 2.0 standard deviations score and/or arm span/height ratio < 0.965. [e]Binder criteria: adjusted extremities-trunk ratio [(calculated subischial leg length + arm span)/sitting height] less than [1.95 + 1/2 height (m)] is altered.[15] %, percentage; BMI, body mass index; CNVs, copy number variations; DLW, dyschondrosteosis de Leri-Weill; ISS, idiopathic short stature; W, birth weight less than −2.0 SDS; L, birth length less than −2.0 SDS; LoF, loss-of-function; NL, individuals without short stature; SDS, standard score deviation; SGA, small for gestational age; SH, sitting height.

Table 2. Possible genetic modifiers of SHOX phenotype identified by targeted gene panel sequencing.

Family[a]	Genomic position[b]	Gene	Location: [Transcript: exon: allelic variant (protein)]	MAF[c]	REVEL[d]	ACMG/AMP[e]
Individuals with gene disruptive mutation						
2	Chr9:35802206	*NPR2*	NM_003995.4:exon10:c.1636A > T (p.Asn546Tyr)	0.00002845	0.57	VUS (PM1, PM2, PP5)
12	Chr6:45480010	*RUNX2*	NM_001024630.4:exon7:c.887C > T (p.Pro296Leu)	0.0004	0.67	VUS (PM2, PP2,PP3, BP6)
17	Chr10:94825984	*CYP26C1*	NM_183374.3:exon5:c.1133G > A (p.Arg378His)	0.0004	0.96	LP (PS3, PM2, PP3, BP1)
23	Chr17:7578463	*TP53*	NM_000546.6:exon5:c.467G > A (p.Arg156His)	0.0012	0.63	VUS (PM2, PM5, PP2, BS3)
46	Chr15:89401037	*ACAN*	NM_013227.4:exon12:c.5221G > C (p.Gly1741Arg)	0	0.23	VUS (PM2, BP4)
Individuals with CNVs involving regulatory regions						
29	Chr10:94825984	*CYP26C1*	NM_183374.3:exon5:c.1133G > A (p.Arg378His)	0.0004	0.96	LP (PS3, PM2, PP3, BP1)
34	Chr15:89401055	*ACAN*	NM_013227.4:exon12:c.5239_5248del (p.Gly1747LeufsTer3)	0	—	LP (PVS1, PM1)
35	Chr12:28122319	*PTHLH*[f]	Chr12:(?_28122319)_(28123008_?)-DEL	—	—	LP (PVS1, PM1)
37	Chr9:35805542	*NPR2*	NM_003995.4:exon13:c.1922C > T (p.Ser641Leu)	0.00003248	0.47	VUS (PM1, PM2)

[a]Family carrying the allelic variant. [b]Genomic position according to Human Genome Building GCRh37, HG19. [c]MAF, minor allele frequency based on the highest frequency observed in gnomAD (Genome Aggregation Database),[22] ABraOM (Brazilian Online Mutation Archive),[21] and Sela (Laboratorio de Sequenciamento em Larga Escala)[23] database. [d]REVEL, an in silico tool for prediction of pathogenicity of missense variants.[24] [e]Pathogenicity criteria according to ACMG (American College of Medical Genetics and Genomics):[26] LP, likely pathogenic; VUS, variants of uncertain significance. [f]CNV analysis by CONTRA: confirmed and segregated in the family by real-time PCR technique. CNV, copy number variants.

gene (MIM*108961). The proband, which had a milder phenotype, had only the *SHOX* deletion. In family 17, the variant in *CYP26C1* was identified in a patient (II.1) with a *de novo* heterozygous *SHOX* deletion. The patient (II.1) had a more severe body disproportion than is usually seen in children with *SHOX* deficiency but did not have short stature or Madelung's deformity. The other variants were in the Tumor protein p53 (*TP53*) gene (MIM*191170) in family 23, that cosegregated with Madelung's deformity and more severe body disproportion in individual II.2, Runt-related transcription factor 2 (*RUNX2*) (MIM*600211) in family 12, that cosegregated with Madelung's deformity in individual I.1 and Aggrecan (*ACAN*) (MIM*155760) in family 46, that cosegregated with short stature in individual II.1.

Potential genetic modifiers identified in individuals with alterations restricted to the *SHOX* regulatory region

We identified 3 LP variants and 1 VUS in 18 families (10 individuals) with a deletion in the *SHOX* area (Figure 2B, Table 2).

In family 37, the individual with an isolated *SHOX*-area deletion (I.1) had a height in the lower limit of normality while the individual with only an *NPR2* alteration (I.2) and individuals with combined alteration of *SHOX* and *NPR2* (II.1 and III.1) had more severe short stature (height SDS less than −3.0). This was consistent with cosegregation of the *NPR2* variant with short stature in three generations and a low penetrance of an isolated small deletion in the *SHOX* area.

In family 34, in addition to the heterozygous deletion compromising the *SHOX* regulatory region, a likely pathogenic variant was identified in the *ACAN* gene. Both variants were present in the father (I.2) and in the proband (II.2) with short stature without Madelung's deformity and body disproportion. The patient had significant bone age advancement (more than 2 years relative to chronological age) while being prepubertal, a recognized phenotype of *ACAN* haploinsufficiency.

Family 35 had a heterozygous deletion in the *SHOX*-area region. The proband (II.1) also had a heterozygous deletion of 689 base pairs (bp) comprising exons 3 and 4 of the *PTHLH* gene. Deletions comprising this gene have already been associated with Brachydactyly phenotype type E (MIM#613382), characterized by shortening of the third to fifth metacarpal and short stature, a phenotype similar to that presented by the patient (II.1) (shortening of the fourth metacarpal and short stature). The *PHTLH* deletion was inherited from the father (I.2) who was of normal height, but with apparent/mild brachydactyly, and the *SHOX* mutation was inherited from the mother (I.1) who was of normal height, but with a disproportionate body. In family 17, a likely pathogenic *CYP26C1* variant was found in addition to the heterozygous *SHOX* regulatory region deletion in an individual with short stature and Madelung's deformity. Family information was not available.

Prevalence of a second variant with a potential role as a genetic modifier of the phenotype in *SHOX* deficiency

Families with *SHOX* alterations restricted to the regulatory region had a higher prevalence of a second likely pathogenic variant with a potential role as a genetic modifier than families with an alteration in *SHOX* coding regions (2.9% and 27%, respectively, $P = .04$) (Figure 1).

Discussion

A wide phenotypic variability may be seen among individuals with *SHOX* deficiency, even among members of the same family carrying an identical gene alteration.[2] Several factors can influence the phenotypic expression, including environmental, epigenetic, polygenic, and digenic factors. In the present study, we analyzed several families with *SHOX* haploinsufficiency to assess genetic factors that can influence phenotype variability. We evaluated the presence of rare cis and trans variants in *SHOX* locus along with the causative mutation, as well as variants in genes associated with growth disorders or involved in *SHOX* activity. Noteworthy, we identified a second genetic cause of short stature that segregated with more severe phenotypes in 4 of 48 families. This finding was even more prevalent

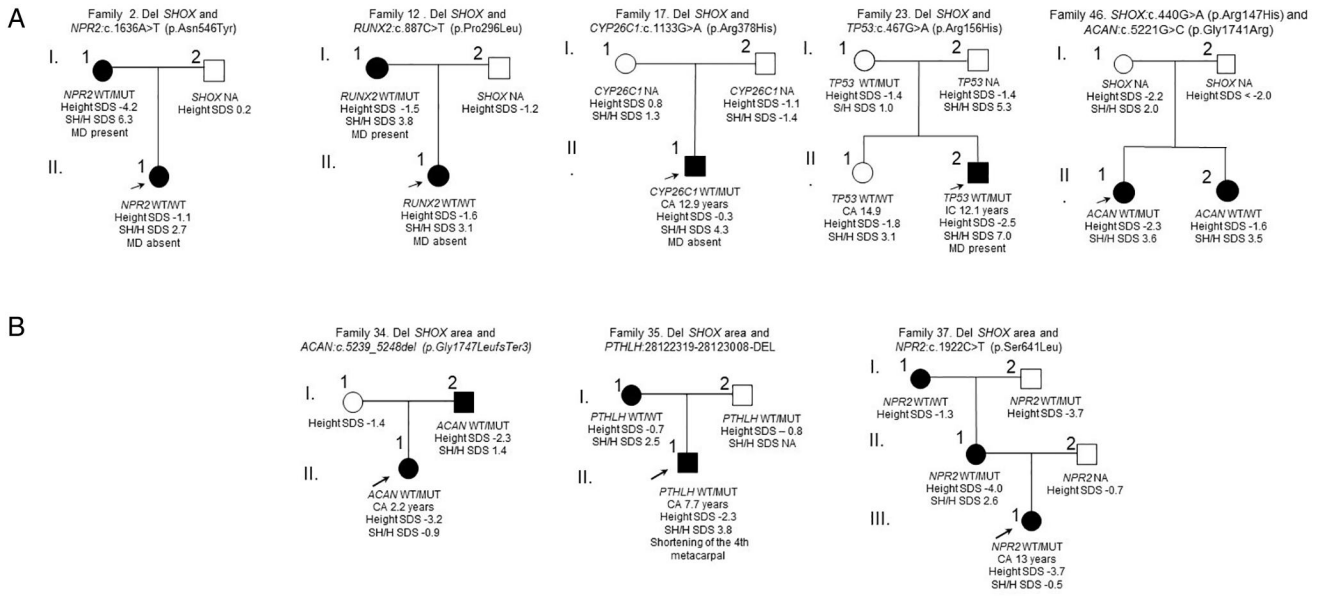

Figure 2. Pedigrees of the families with *SHOX* deficiency and a second variant with a potential role as a genetic modifier. Individuals with (A) alterations encompassing *SHOX* coding region (Del *SHOX*) and (B) individuals with alterations restricted to *SHOX* regulatory region (Del *SHOX* area). Black circles and rectangles: females and males with *SHOX* deficiency, respectively. CA, chronological age; MD, Madelung's deformity; MUT, mutation; NA, not available; SDS, standard deviation score; SH/H, sitting height/height; WT, wild type.

in families with deletions restricted to the *SHOX* regulatory region, where a second likely pathogenic gene alteration was found in 27% of the families analyzed (3 of 11 families).

To date, only one study has investigated the presence of other gene variants modulating the phenotype in individuals with *SHOX* deficiency. Montalbano *et al.*[12] analyzed 69 families with *SHOX* deficiency with a large phenotypic variability among the individuals. They identified variants in the *CYP26C1* gene, which segregated with the most severe phenotypes in three families. Del Pino *et al.*[27] performed Sanger sequencing of *CYP26C1* in 30 individuals (19 probands and 11 first-degree relatives, total of 15 families) with *SHOX* deficiency, but no mutation in *CYP26C1* was identified. In contrast, we identified a previously described *CYP26C1* variant (c.1133G > A:p.Arg378His), in 2 of 48 analyzed families. This likely pathogenic variant has a negative impact on RA metabolism,[12] and it was associated with a more adverse phenotype, supporting the role of *CYP26C1* as a phenotypic modulator in individuals with *SHOX* deficiency.

In addition to *CYP26C1* alterations, we also identified variants of interest in *NPR2*, *RUNX2*, *ACAN*, *TP53*, and *PTHLH* genes in more affected individuals from families with *SHOX* defects. These gene pathways overlap with *SHOX's* in the growth plate and were associated with short stature phenotype with or without skeletal dysmorphisms.[28–32]

Two *NPR2* variants were identified in patients with *SHOX* deficiency and more severe phenotypes. *NPR2* gene encodes a guanylate cyclase receptor important for endochondral bone growth, the NPR-B.[33,34] Patients with heterozygous pathogenic variants in the *NPR2* gene have short stature with nonspecific skeletal findings (OMIM#616255)[29] and may have phenotypes overlapping with Léri-Weill dyschondrosteosis (OMIM#127300).[28] Previous studies demonstrated that the *NPR2*:c.1636A > T (p.Asn546Tyr) variant causes an impairment in receptor activity with moderate reduction in cGMP synthesis.[28] This hypomorphic variant alone may not be sufficient to influence phenotype but in association with *SHOX* haploinsufficiency can lead to a more severe phenotype as observed in individuals who carry both alterations (Figure 2A, Family 2). The variant *NPR2*: c.1922C > T (p.Ser641Leu) has not been previously reported, and it is located in an intracellular kinase homology domain that can lead to an impaired ligand-induced guanylate cyclase activity.[35] This variant cosegregated with short stature in three generations and when associated with a *SHOX* alteration led to a more severe short stature. Additionally, the *NPR2* variant observed in this family (Figure 2B, Family 37) has a greater contribution to the short stature phenotype than does the *SHOX*-area alteration.

SHOX modulates the expression levels of *RUNX2*, required for the differentiation (maturation) of prehypertrophic chondrocytes to hypertrophic chondrocytes.[4,36] Heterozygous *RUNX2* mutations lead to Cleidocranial dysplasia (CCD) (OMIM#119600).[30] A common mechanism of disease is the presence of missense variants in the Runt-domain responsible for DNA binding.[30] In addition, common variants in the *RUNX2* gene are related to height in genome-wide association studies.[37] The missense variant identified in the present study, c.887C > T (p.Pro296Leu), is not located in the main RUNX2 domain, but in a proline/serine/threonine rich region essential for transcriptional activation of target genes where loss-of-function variants cause CCD.[30] *SHOX* and *RUNX2* genes are important in the regulation of chondrocyte differentiation in the growth plate and they are part of the same pathway. The *SHOX* haploinsufficiency phenotype can be accentuated by a hypomorphic variant in the *RUNX2* gene, since it disrupts the residual effect of the remaining copy of *SHOX* leading to a more severe phenotype. In the case of family 12 (Figure 2A), Madelung's deformity is only seen in individuals with the two variants.

SHOX interacts with the *SOX* trio SOX5, SOX6, and SOX9.[38] SOX9 regulates the transcription of multiple genes

in the proliferative zone, including *ACAN*.[39] Different *SHOX* missense mutations fail to interact with the *SOX* trio[38] and could disrupt normal transcription of the *ACAN* gene. *ACAN* encodes a proteoglycan-aggrecan protein that is an essential component of the extracellular matrix (ECM) in cartilaginous development in the growth plate, producing a hydrated gel structure.[40] Heterozygous mutations in *ACAN* are responsible for spondyloepimetaphyseal dysplasia, Kimberley type (MIM#608361) or short stature with advanced bone age (MIM#165800).[40] The affected member of family 46 (Figure 2A) was heterozygous for a missense mutation in *ACAN*: p.Gly1741Arg and had a more severe short stature phenotype than his brother with isolated *SHOX* alteration. This variant is located in exon 12 which encodes the second interglobular domain in the chondroitin sulfate (CS) attachment region. This CS region fixes negative charges that attract positive ions and water to the ECM, producing a hydrodynamic effect in cartilage.[40] Thus, mutations in genes involved in CS synthesis and sulfation can cause skeletal dysplasias with different degrees of severity.[40] Additionally, in family 34 (Figure 2B), we identified a frameshift mutation in *ACAN*:p.Gly1747LeufsTer3. Frameshift mutations mapping closely upstream (p.Gly1330TrpfsTer221) and downstream (p.Gly1797GlyfsTer52) have already been associated with spondyloepiphyseal dysplasia, Kimberly type[41] and short stature with advanced bone age,[31] respectively.

The *PTHLH* gene has 6 exons and encodes a protein that binds to the PTH receptor in pre hypertrophic chondrocytes in the growth plate.[42] This binding preserves the proliferative chondrocyte and prevents its differentiation into hypertrophic chondrocytes.[42] *PTHLH* expression is under control of *IHH*, *SOX9*, and *RUNX2* genes,[42] which have an interactive signaling pathway with *SHOX* gene. Disruptive *PTHLH* mutations are associated with the phenotype of brachydactyly type E (MIM*113300).[32] The *PTHLH* intragenic deletion described in our proband (family 35, II.1) is close to other described deletions and haploinsufficiency is a common mechanism of disease involving this gene.[32] Both *PTHLH* and *SHOX* haploinsufficiency have variable intrafamilial phenotypic features. In this family, the mother (I.1) with *SHOX* alteration restricted to the regulatory region had isolated body disproportion and the father (I.2) with a *PTHLH* mutation has apparent brachydactyly without other findings. The index case has an overlapping phenotype resulting from the presence of both alterations.

Based on our results, we hypothesize that the presence of a second variant in growth plate-related genes may intensify the dysregulation of chondrocytes in the growth plate zones related to a primary alteration in the *SHOX* gene. This leads to a more severe phenotype than that present in individuals with a solo *SHOX* alteration. This effect is more evident in individuals with defects in the *SHOX* regulatory region, who typically have a milder phenotype, presenting a lower degree of body disproportion and/or Madelung's deformity, when compared to alterations directly involving the *SHOX* coding region.[13,43,44] Deletions in *SHOX* regulatory regions lead to a decrease in *SHOX* expression[45] and are associated with low penetrance of the phenotype.[44] In our series, 27% of the families with isolated alterations in the *SHOX* regulatory region had a second variant. We propose that the presence of a second variant is important to determine the penetrance and expression of the phenotype in individuals with isolated alterations in the regulatory region, characterizing a digenic or oligogenic inheritance.

Individuals with DLW or children with ISS[14,46] are investigated for *SHOX* deficiency by MLPA followed by Sanger sequencing.[47] Most of the time, individuals with a positive finding do not undergo further genetic testing. Thus, this candidate gene approach may prevent the identification of other variants that can contribute to the patient's phenotype. Despite being based on a relatively small number of families and patients, our study sheds light on the issue of co-occurrence of a second genetic alteration in patients with *SHOX* deficiency. We identified possible modifying elements even using a panel analysis with a limited number of genes. These findings might be even more frequent using a WES approach. Additionally, future functional studies are necessary to better understand the interaction of *SHOX* and other genes during skeletogenesis and growth plate pathways.

In conclusion, variants in genes related to the growth plate have a potential role as phenotypic modifiers in individuals with *SHOX* deficiency. In individuals with a *SHOX* alteration restricted to the regulatory region, this alteration could be critical to determining the penetrance and expression of the phenotype.

Statements

Statement of ethics

Study approval statement: This study protocol was approved by the Research Ethics Committee of the University of Sao Paulo Medical School General Hospital (approval number CAAE-37868114.3.0000.0068).

Consent to participate statement: Parental consent for molecular analysis and clinical information assessment was obtained after full explanation of the purpose of this study. This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

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Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

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Contributors

N.C.B.D and M.F.A.F made substantial contributions to acquisition and analysis of data and helped draft the manuscript. A.M.L. made substantial contributions to the bioinformatic

analysis of data for the manuscript. N.L.M.A., R.C.R., L.P.C, C.A., and L.G.C. made substantial contributions to acquisition of data. R.C.S. made substantial contributions to acquisition and revision of data and their critical interpretation. I.J.P.A. and B.B.M. made substantial contributions to the conception of the work and provided critical revisions. A.A.L.J. made substantial contributions to the conception of the work, acquisition, analysis, and interpretation of data, and helped draft the manuscript. All authors approved the final submitted version and agreed to be accountable for the accuracy and integrity of the work.

Conflict of interest: A.A.L.J. has received speaker fees from Novo Nordisk and Pfizer, has independent research grant from BioMarin and has received consulting fees from Novo Nordisk. The other authors declare that they have no competing financial interest to declare.

Data availability

The datasets generated and/or analyzed during this study are not publicly available but are available from the corresponding author upon reasonable request.

References

1. Ellison JW, Wardak Z, Young MF, Gehron Robey P, Laig-Webster M, Chiong W. PHOG, a candidate gene for involvement in the short stature of turner syndrome. *Hum Mol Genet*. 1997;6(8):1341-1347. https://doi.org/10.1093/hmg/6.8.1341
2. Rao E, Weiss B, Fukami M, *et al*. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nat Genet*. 1997;16(1):54-63. https://doi.org/10.1038/ng0597-54
3. Marchini A, Marttila T, Winter A, *et al*. The short stature homeodomain protein SHOX induces cellular growth arrest and apoptosis and is expressed in human growth plate chondrocytes. *J Biol Chem*. 2004;279(35):37103-37114. https://doi.org/10.1074/jbc.M307006200
4. 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. https://doi.org/10.1210/er.2016-1036
5. Binder G, Schwarze CP, Ranke MB. Identification of short stature caused by SHOX defects and therapeutic effect of recombinant human growth hormone. *J Clin Endocrinol Metab*. 2000;85(1):245-249. https://doi.org/10.1210/jcem.85.1.6375
6. Blum WF, Crowe BJ, Quigley CA, *et al*. Growth hormone is effective in treatment of short stature associated with short stature homeobox-containing gene deficiency: two-year results of a randomized, controlled, multicenter trial. *J Clin Endocrinol Metab*. 2007;92(1):219-228. https://doi.org/10.1210/jc.2006-1409
7. Jorge AAL, Souza SC, Nishi MY, *et al*. SHOX mutations in idiopathic short stature and Leri-Weill dyschondrosteosis: frequency and phenotypic variability. *Clin Endocrinol (Oxf)*. 2007;66(1):130-135. https://doi.org/10.1111/j.1365-2265.2006.02698.x
8. Binder G, Renz A, Martinez A, *et al*. SHOX Haploinsufficiency and Leri-Weill dyschondrosteosis: prevalence and growth failure in relation to mutation, sex, and degree of wrist deformity. *J Clin Endocrinol Metab*. 2004;89(9):4403-4408. https://doi.org/10.1210/jc.2004-0591
9. Belin V, Cusin V, Viot G, *et al*. SHOX mutations in dyschondrosteosis (Leri-Weill syndrome). *Nat Genet*. 1998;19(1):67-69. https://doi.org/10.1038/ng0198-67
10. Leri A, Weill J. Une affection congenitale et symetrique du developpement osseux : la dyschondrosteose. *Bull Mem Soc Med HopParis*. 1929;35:1491-1494.
11. Ross JL, Scott CJ, Marttila P, *et al*. Phenotypes associated with SHOX deficiency. *J Clin Endocrinol Metab*. 2001;86(12):5674-5680. https://doi.org/10.1210/jcem.86.12.8125
12. Montalbano A, Juergensen L, Roeth R, *et al*. Retinoic acid catabolizing enzyme CYP26C1 is a genetic modifier in SHOX deficiency. *EMBO Mol Med*. 2016;8(12):1455-1469. https://doi.org/10.15252/emmm.201606623
13. Rosilio M, Huber-Lequesne C, Sapin H, Carel J-C, Blum WF, Cormier-Daire V. Genotypes and phenotypes of children with SHOX deficiency in France. *J Clin Endocrinol Metab*. 2012;97(7):E1257-E1265. https://doi.org/10.1210/jc.2011-3460
14. Rappold G, Blum WF, Shavrikova EP, *et al*. Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. *J Med Genet*. 2007;44(5):306-313. https://doi.org/10.1136/jmg.2006.046581
15. Binder G, Ranke MB, Martin DD. Auxology is a valuable instrument for the clinical diagnosis of SHOX haploinsufficiency in school-age children with unexplained short stature. *J Clin Endocrinol Metab*. 2003;88(10):4891-4896. https://doi.org/10.1210/jc.2003-030136
16. Kuczmarski RJ, Ogden CL, Guo SS, *et al*. 2000 CDC growth charts for the United States: methods and development. *Vital Health Stat*. 2002;246:1-190.
17. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks. *J Pediatr*. 1969;74(6):901-910. https://doi.org/10.1016/S0022-3476(69)80224-6
18. Gerver WJM, Bruin R. *Paediatric Morphometrics : A Reference Manual*. Datawyse / Universitaire Pers Maastricht, Maastricht, Netherlands; 2001.
19. Szklarczyk D, Gable AL, Nastou KC, *et al*. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res*. 2021;49(D1):D605-D612. https://doi.org/10.1093/nar/gkaa1074
20. 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. https://doi.org/10.1210/jc.2018-01971
21. Naslavsky MS, Yamamoto GL, Almeida TF, *et al*. Exomic variants of an elderly cohort of Brazilians in the ABraOM database. *Hum Mutat*. 2017;38(7):751-763. https://doi.org/10.1002/humu.23220
22. Karczewski KJ, Francioli LC, Tiao G, *et al*. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434-443. https://doi.org/10.1038/s41586-020-2308-7
23. Lerario AM, Mohan DR, Montenegro LR, *et al*. SELAdb: A database of exonic variants in a Brazilian population referred to a quaternary medical center in São Paulo. *Clinics*. 2020;75:e1913. https://doi.org/10.6061/clinics/2020/e1913
24. Ioannidis NM, Rothstein JH, Pejaver V, *et al*. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. *Am J Hum Genet*. 2016;99(4):877-885. https://doi.org/10.1016/j.ajhg.2016.08.016
25. Li J, Lupat R, Amarasinghe KC, *et al*. CONTRA: copy number analysis for targeted resequencing. *Bioinformatics*. 2012;28(10):1307-1313. https://doi.org/10.1093/bioinformatics/bts146
26. Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. https://doi.org/10.1038/gim.2015.30
27. Del Pino M, Aza-Carmona M, Medino-Martín D, *et al*. SHOX deficiency in Argentinean cohort: long-term auxological follow-up and a family's new mutation. *J Pediatr Genet*. 2019;8(03):123-132. https://doi.org/10.1055/s-0039-1691788
28. 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. https://doi.org/10.1210/jc.2015-1612
29. Vasques GA, Amano N, Docko AJ, *et al*. Heterozygous mutations in natriuretic peptide receptor-B (NPR2) gene as a cause of short stature in patients initially classified as idiopathic short stature. *J Clin Endocrinol Metab*. 2013;98(10):E1636-E1644. https://doi.org/10.1210/jc.2013-2142
30. Jaruga A, Hordyjewska E, Kandzierski G, Tylzanowski P. Cleidocranial dysplasia and RUNX2-clinical phenotype-genotype correlation. *Clin Genet*. 2016;90(5):393-402. https://doi.org/10.1111/cge.12812
31. Quintos JB, Guo MH, Dauber A. Idiopathic short stature due to novel heterozygous mutation of the aggrecan gene. *J Pediatr Endocrinol Metab*. 2015;28(7-8):927-932. https://doi.org/10.1515/jpem-2014-0450
32. Klopocki E, Hennig BP, Dathe K, *et al*. Deletion and point mutations of PTHLH cause brachydactyly type E. *Am J Hum Genet*. 2010;86(3):434-439. https://doi.org/10.1016/j.ajhg.2010.01.023
33. Schulz S. C-type natriuretic peptide and guanylyl cyclase B receptor. *Peptides*. 2005;26(6):1024-1034. https://doi.org/10.1016/j.peptides.2004.08.027
34. Marchini A, Häcker B, Marttila T, *et al*. BNP Is a transcriptional target of the short stature homeobox gene SHOX. *Hum Mol Genet*. 2007;16(24):3081-3087. https://doi.org/10.1093/hmg/ddm266
35. Hachiya R, Ohashi Y, Kamei Y, *et al*. Intact kinase homology domain of natriuretic peptide receptor-B is essential for skeletal development. *J Clin Endocrinol Metab*. 2007;92(10):4009-4014. https://doi.org/10.1210/jc.2007-1101
36. Enomoto H, Enomoto-Iwamoto M, Iwamoto M, *et al*. Cbfa1 is a positive regulatory factor in chondrocyte maturation. *J Biol Chem*. 2000;275(12):8695-8702. https://doi.org/10.1074/jbc.275.12.8695
37. Kichaev G, Bhatia G, Loh P-R, *et al*. Leveraging polygenic functional enrichment to improve GWAS power. *Am J Hum Genet*. 2019;104(1):65-75. https://doi.org/10.1016/j.ajhg.2018.11.008
38. Aza-Carmona M, Shears DJ, Yuste-Checa P, *et al*. SHOX interacts with the chondrogenic transcription factors SOX5 and SOX6 to activate the aggrecan enhancer. *Hum Mol Genet*. 2011;20(8):1547-1559. https://doi.org/10.1093/hmg/ddr032
39. Hattori T, Müller C, Gebhard S, *et al*. SOX9mis a major negative regulator of cartilage vascularization, bone marrow formation and endochondral ossification. *Development*. 2010;137(6):901-911. https://doi.org/10.1242/dev.045203
40. Aspberg A. The different roles of aggrecan interaction domains. *J Histochem Cytochem*. 2012;60(12):987-996. https://doi.org/10.1369/0022155412464376
41. Gleghorn L, Ramesar R, Beighton P, Wallis G. A mutation in the variable repeat region of the aggrecan gene (AGC1) causes a form of spondyloepiphyseal dysplasia associated with severe, premature osteoarthritis. *Am J Hum Genet*. 2005;77(3):484-490. https://doi.org/10.1086/444401
42. Kronenberg HM. PTHrp and skeletal development. *Ann N Y Acad Sci*. 2006;1068(1):1-13. https://doi.org/10.1196/annals.1346.002
43. Kant SG, Broekman SJ, de Wit CC, *et al*. Phenotypic characterization of patients with deletions in the 3′-flanking SHOX region. *PeerJ*. 2013;1:e35. https://doi.org/10.7717/peerj.35
44. Benito-Sanz S, Royo JL, Barroso E, *et al*. Identification of the first recurrent PAR1 deletion in Léri-Weill dyschondrosteosis and idiopathic short stature reveals the presence of a novel SHOX enhancer. *J Med Genet*. 2012;49(7):442-450. https://doi.org/10.1136/jmedgenet-2011-100678
45. Fukami M, Kato F, Tajima T, Yokoya S, Ogata T. Transactivation function of an ~800-bp evolutionarily conserved sequence at the SHOX 3′ region: implication for the downstream enhancer. *Am J Hum Genet*. 2006;78(1):167-170. https://doi.org/10.1086/499254
46. Malaquias AC, Scalco RC, Fontenele EGP, *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. https://doi.org/10.1159/000355411
47. Funari MFA, de Barros JS, Santana LS, *et al*. Evaluation of SHOX defects in the era of next-generation sequencing. *Clin Genet*. 2019;96(3):261-265. https://doi.org/10.1111/cge.13587

Supplemental Material: Identification of a second genetic alteration in patients with *SHOX* deficiency: a potential explanation for phenotype variability

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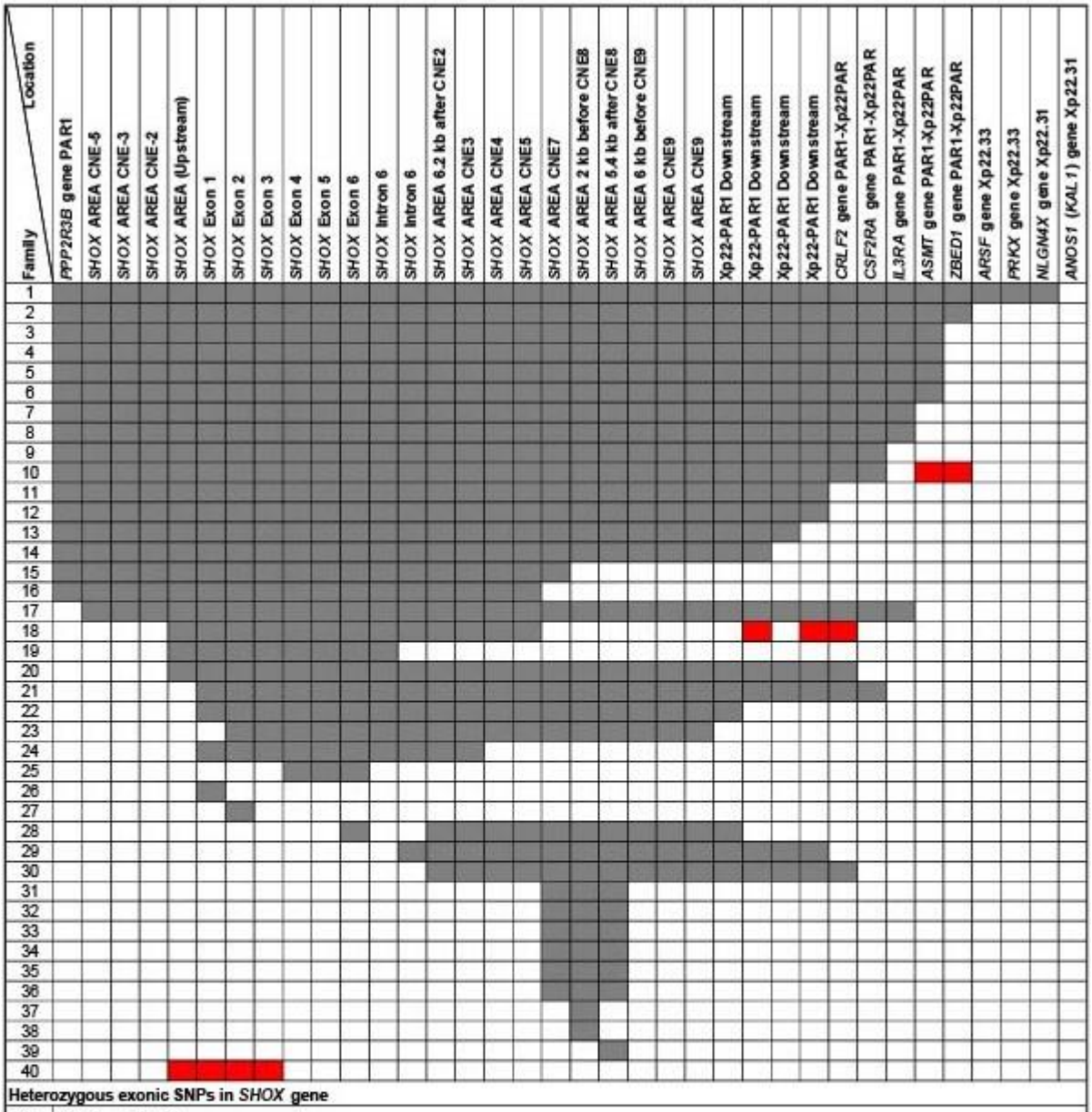
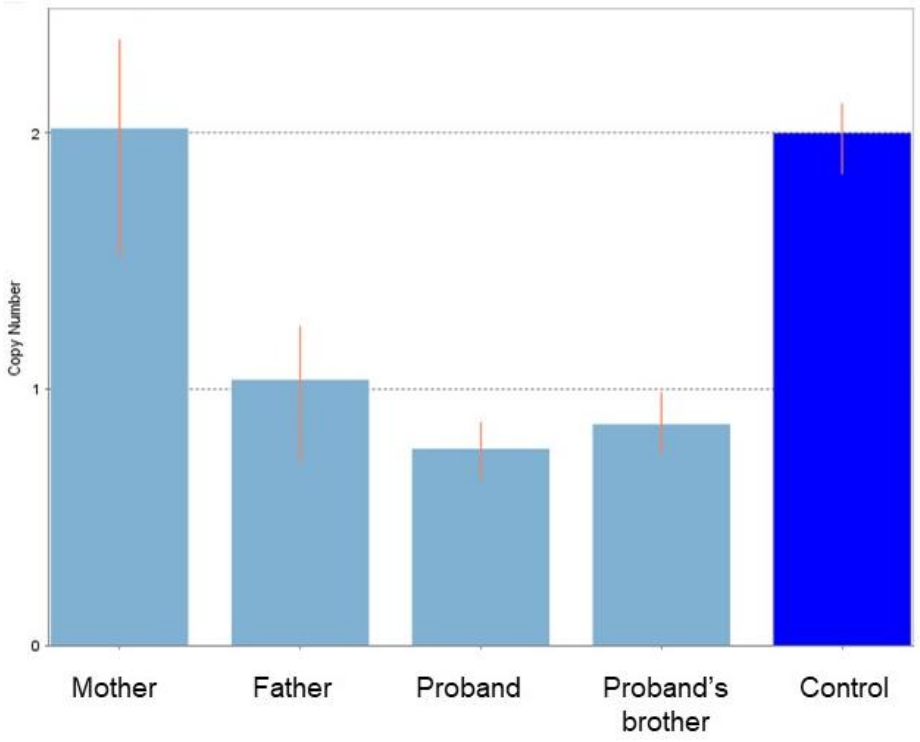
Supplemental Figure 1: Scheme of *SHOX* gene defects in the 48 analyzed families. On the top, the representation of the MLPA probes, on the left are the families, and on the right, their respective SHOX gene defect. The first 40 families have copy number variations (CNVs) in the *SHOX* gene: the white boxes represent the preserved regions, the gray boxes are the heterozygous deletion, and the red boxes are the heterozygous duplication of the MLPA probes. Families 41 to 48 have single nucleotide variants (SNVs) in the *SHOX* gene. PAR1: pseudoautosomal region 1.



Supplemental Figure 2: Results generated by the Taqman™ Copy Number Assay (Applied Biosystems, Waltham, MA) with CopyCaller Software v2.1 (Thermo Fisher

Scientific Inc, Waltham, MA) used to confirm the parathyroid hormone-like hormone (*PTHLH*) gene deletion (probe: Chr12: Hs03005427_cn; catalog number: 4400291). Copy number analysis: the dark blue column represents a negative control, and the light blue columns represent the individuals from family 35. The mother had two copies of the *PTHLH* gene, while the proband, the father and the proband's brother were missing one copy confirming the *PTHLH* deletion.



Supplemental Table 1. Clinical scoring system to identify short stature individuals for *SHOX* analysis according to Rappold criteria.

Score item	Criterion	Score points
Arm span/height ratio	<96.5%	2
Sitting height/height ratio	>55.5%	2
Body–mass index	>50th percentile	4
Cubitus valgus	Yes	2
Short forearm	Yes	3
Bowing of forearm	Yes	3
Appearance of muscular hypertrophy	Yes	3
Dislocation of ulna (at elbow)	Yes	5
Total		24

Adapted from Rappold et al (Rappold et al., 2007).

Supplemental Table 2. Genes and *SHOX* genomic regions included in the customized target panel to evaluate the presence of genetic modifiers of the phenotype in individuals with *SHOX* deficiency.

Genes in the same network of the *SHOX* gene
ADAMTSL3
BGLAP
BMP4
BMP5
CDKN1A
CDKN1B
COL10A1
CSNK2A1
CTGF
CTSB
EXT1
FBN3
FBXO41
FGF1
FGF18
FGF9
FOXA2
GALNT2
GPR133
HAPLN1
HIF1A
IBSP
INTS1
MAF
MATN1
MEF2C
MMP13
MMP14
MMP8
MMP9
MRGPRG
NCAM1
NKX3-2
NPR3
OGN
PBX1
PQLC2
PTH1R
PTHLH
QRICH1
RBL2
RUNX3
SIRT6
SLC26A2
SPP1
TBX4
TP53
VEGFA
WASHC3
WNT3A
ZFHX3

ZNF521
Genes in the retinoic acid pathway
ALDH1A2
CARM1
CRABP1
CRABP2
CYP26A1
CYP26B1
CYP26C1
RARA
RARB
RARG
RBP1
RDH10
RORA
RORB
RXRA
RXRB
RXRG
STRA6
Genes related to growth plate and short stature
ACAN
ADAMTS10
ADAMTS17
BMP2
BMPR1B
CBL
CCDC8
COL2A1
COMP
CUL7
*FBN1*_ex41-43
FGF8
FGFR1
FGFR3
GDF5
GH1 region
GHR
*GHR*_IVS6
GHSR
GNAS
HDAC6
HOXA9
IGF1
IGF1R
IGFALS
IHH
KAL1
LZTR1
NF1
NPPB
NPPC

NPR2
OBSL1
PRKG2
PTPN11
ROR2
RUNX2
SCUBE3
SHOX2
SOX5
SOX6
SOX9
STAT5B
WNT5A
SHOX genomic region
chrx:580000-621000
SHOX enhancers (AREA)
chrx:398100-399050
chrx:460100-460900
chrx:516400-517400
chrx:579800-581100
chrx:674500-675500
chrx:694500-695500
chrx:713900-714900
chrx:750700-752300
chrx:763900-764900
chrx:780400-781400
chrx:800700-802000
chrx:809000-810000
chrx:817000-818400
chrx:828000-829000
chrx:834500-835700
chrx:850080-851150
chrx:898800-899890
chrx:963150-964300
chrx:1029150-1030300
chrx:1327150-1328300
Pseudoautosomal region 1
chrx:83079-84079
chrx:183079-184079
chrx:283079-284079
chrx:383079-384079
chrx:483079-484079
chrx:707558-708558
chrx:807558-808558
chrx:907558-908558
chrx:1007558-1008558
chrx:1107558-1108558
chrx:1207558-1208558

chrx:1307558-1308558
chrx:1407558-1408558
chrx:1507558-1508558
chrx:1607558-1608558
chrx:1707558-1708558
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chrx:2207558-2208558
chrx:2307558-2308558
chrx:2407558-2408558
chrx:2507558-2508558
chrx:2607558-2608558
chrx:2707558-2708558
SHOX Flanking genes
ARSF
ASMT
CRLF2
FANCB
GTPBP6
NLGN4X
PLCXD1
PPP2R3B
PRKX
STS

The sequences were aligned with the human reference assembly (UCSC hg 19, GRCh37).

Reference

Rappold, G., Blum, W. F., Shavrikova, E. P., Crowe, B. J., Roeth, R., Quigley, C. A., Ross, J. L., & Niesler, B. (2007). Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. *Journal of Medical Genetics*, *44*(5), 306 LP – 313. https://doi.org/10.1136/jmg.2006.046581

3.3 CAPÍTULO 2: ADULT HEIGHT OF PATIENTS WITH *SHOX* HAPLOINSUFFICIENCY WITH OR WITHOUT GH THERAPY: A REAL-WORLD SINGLE-CENTER STUDY

Dantas NCB, Funari MFA, Vasques GA, Andrade NLM, Rezende RC, Brito V, Scalco RC, Arnhold IJP, Mendonca BB, Jorge AAL. Adult height of patients with SHOX haploinsufficiency with or without GH therapy: a real-world single-center study. Horm Res Paediatr. 2022;95(3):264-274. doi: 10.1159/000524374.

Nos últimos anos houve um crescimento contínuo de indivíduos acompanhados no nosso serviço devido à baixa estatura decorrente da haploinsuficiência do *SHOX*. Durante esses anos, podemos acompanhar o crescimento desses pacientes até atingirem a altura adulta. Previamente, publicamos os resultados desse seguimento com uma casuística menor[59]. Neste estudo, expandimos a casuística e avaliamos a efetividade do tratamento com hormônio do crescimento para o ganho de altura adulta comparando um grupo tratado com um grupo não-tratado com hormônio do crescimento usando evidências de mundo real.

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Adult Height of Patients with *SHOX* Haploinsufficiency with or without GH Therapy: A Real-World Single-Center Study

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Keywords
SHOX deficiency · Short stature · Growth hormone therapy · Gonadotropin-releasing hormone agonist · Adult height

Abstract
Introduction: Isolated *SHOX* haploinsufficiency is a common monogenic cause of short stature. Few studies compare untreated and rhGH-treated patients up to adult height (AH). Our study highlights a growth pattern from childhood to AH in patients with *SHOX* haploinsufficiency and analyzes the real-world effectiveness of rhGH alone or plus GnRH analog (GnRHa). ***Methods:*** Forty-seven patients (18 untreated and 29 rhGH-treated) with *SHOX* haploinsufficiency were included in a longitudinal retrospective study. Adult height was attained in 13 untreated and 18 rhGH-treated (rhGH alone [*n* = 8] or plus GnRHa [*n* = 10]) patients. ***Results:*** The untreated group decreased height SDS from baseline to AH (−0.8 [−1.1; −0.4]), with an increase in the prevalence of short stature from 31% to 77%. Conversely, the rhGH-treated group had an improvement in height SDS from baseline to AH (0.6 [0.2; 0.6]; $p < 0.001$), with a reduction in the prevalence of short stature (from 61% to 28%). AH in the rhGH-treated patients was 1 SD (6.3 cm) taller than in untreated ones. Regarding the use of GnRHa, the subgroups (rhGH alone or plus GnRHa) attained similar AH, despite the higher prevalence of pubertal patients and worse AH prediction at the start of rhGH treatment in patients who used combined therapy. ***Conclusion:*** The use of rhGH treatment improves AH in patients with *SHOX* haploinsufficiency, preventing the loss of height potential during puberty. In peripubertal patients, the addition of GnRHa to rhGH allows AH attainment similar to the AH of patients who start rhGH alone in the prepubertal age.

Introduction

The short stature homeobox-containing (*SHOX*) gene ([Mendelian Inheritance in Man] MIM *312865) is located in the short arm pseudoautosomal region (PAR1) of the sex chromosomes and escapes from X inactivation, requiring two copies of the gene for its normal function [1, 2]. SHOX protein is very important for the normal development of the growth plate. It is mainly expressed in hypertrophic chondrocytes and acts as a transcriptional factor that regulates cell cycle arrest and apoptosis [3, 4].

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Isolated *SHOX* haploinsufficiency is the most important monogenic cause of short stature [3]. Patients with Turner syndrome have total or partial loss of one copy of the X chromosome including *SHOX*. *SHOX* deficiency is thought to contribute for the short stature and skeletal abnormalities of Turner syndrome [3]. Heterozygous mutations in *SHOX* or its regulatory regions are identified in 1–10% of children classified as having "idiopathic" short stature (MIM #300582) [1, 5, 6]. Additionally, similar molecular defects are identified in 70–90% of individuals with Leri-Weill dyschondrosteosis (MIM #127300) [7–9], which is characterized by the presence of disproportionate short stature, mesomelia, and Madelung deformity [10].

Children with isolated *SHOX* haploinsufficiency may involve variable degrees of growth impairment. Longitudinal follow-up studies suggest that growth impairment starts early in life with a birth length in the lower range of normality [8, 11–13]. During the prepubertal period, height is frequently below the 3rd centile in the growth chart with a relatively well-preserved prepubertal growth [11, 13]. Nonetheless, these patients usually have an attenuated pubertal growth spurt with a frequent worsening of height standard deviation scores (SDS) [13–15], usually reaching an average untreated adult height SDS of −2.5 [8, 13, 14, 16–18]. The loss of growth potential observed in these patients suggests that an additional effect of estrogens in the presence of *SHOX* haploinsufficiency leads to the premature fusion of the growth plate [13–15, 17, 18].

Recombinant human growth hormone (rhGH) treatment has been used to improve the adult height of patients with isolated *SHOX* haploinsufficiency. There are few studies evaluating the long-term effect of rhGH treatment [11, 19–23], none of them comparing adult height of *SHOX* patients followed with or without treatment. We previously reported the adult height in 10 patients with *SHOX* defects, in which half of them were treated with rhGH plus GnRH analog (GnRHa) treatment [15]. We observed that the combined therapy in peripubertal children with *SHOX* defects can attenuate the loss of the growth potential during puberty [15].

The present study reports a single-center experience on rhGH therapy in children with isolated *SHOX* haploinsufficiency and analyzes the real-world results of rhGH treatment (with or without puberty modulators [PMs]) on adult height, comparing them with those *SHOX* patients followed without intervention. Our work adds additional evidence regarding the benefits of rhGH treatment in adult height in this group of children.

Materials and Methods

Subjects

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Hospital das Clinicas Ethics Committee (approval number CAAE-37868114.3.0000.0068). Parents (or legal guardians) provided written informed consent after full explanation of the purpose of this study. We retrospectively assessed growth data of children with isolated *SHOX* haploinsufficiency followed between 1995 and 2020 in a single tertiary pediatric endocrinology center. The inclusion criteria were: (1) presence of a heterozygous defect in *SHOX* – all patients were evaluated by multiplex ligation-dependent probe amplification and sequencing methodologies (Sanger or massive parallel sequencing techniques) as previously published [24, 25]; (2) no evidence of organic disorder or other cause of growth impairment, including hypothyroidism and GH deficiency; and (3) enough data to assess childhood growth. Fifty-six children with *SHOX* defects were analyzed. Nine patients were excluded: one due to type 1 diabetes mellitus, one because of adrenal congenital hyperplasia, and 7 cases on account of missing data ($n = 7$). We retained 4 patients without data of the first year of follow-up (3 in the untreated group and 1 in the treated group) due to the availability of adult height data. Therefore, 47 cases were eligible for the present study. Twenty-nine of these children were treated with rhGH, whereas 18 did not receive rhGH therapy for different reasons: absence of short stature at the initial follow-up ($n = 10$), refusal of rhGH therapy ($n = 4$), or late molecular genetic diagnosis ($n = 4$). This group represented an observational control to characterize the natural growth pattern of children harboring *SHOX* alterations. Among the treated patients, puberty was postponed in 11 patients by GnRH analogs use (GnRHa, 2 males and 9 females). Additionally, two boys were treated with aromatase inhibitors (AI, anastrozole, $n = 2$) to slow bone age advancement and allow the progression of secondary sex characteristics. Adult height was reached by 13 patients in the untreated group and by 18 patients in the treated group (8 treated with rhGH alone and 10 with rhGH plus GnRHa). Ten patients were previously described, five from each group [15]. None of the two boys treated with AI achieved adult height at the end of the study.

Study Protocol

The data were systematically collected from medical records. The following data were analyzed: parents' heights, gestational age at birth, birth weight, birth length, chronological age, and the anthropometric data measured at baseline and every 4 months for patients on rhGH treatment and every 6–12 months for patients without treatment. The evaluations included measurements of height, weight, body mass index (BMI), sitting height (SH), armspan, pubertal assessment, and presence of clinical Madelung deformity. Height was measured on a stadiometer graduated in millimeters, weight was measured on a digital scale, and the pubertal stage was assessed according to Tanner stages [26]. Anthropometric data were converted to SDS using age- and gender-specific norms [27–30].

Adult height was defined as the growth velocity equal or inferior to 2 cm/year. IGF-1 was collected at baseline and annually in the treated group until the end of rhGH treatment. IGF-1 levels were determined by different assays and converted to SDS according to the reference method value. Left hand and wrist x-rays were obtained at baseline and annually. Bone age was assessed by two

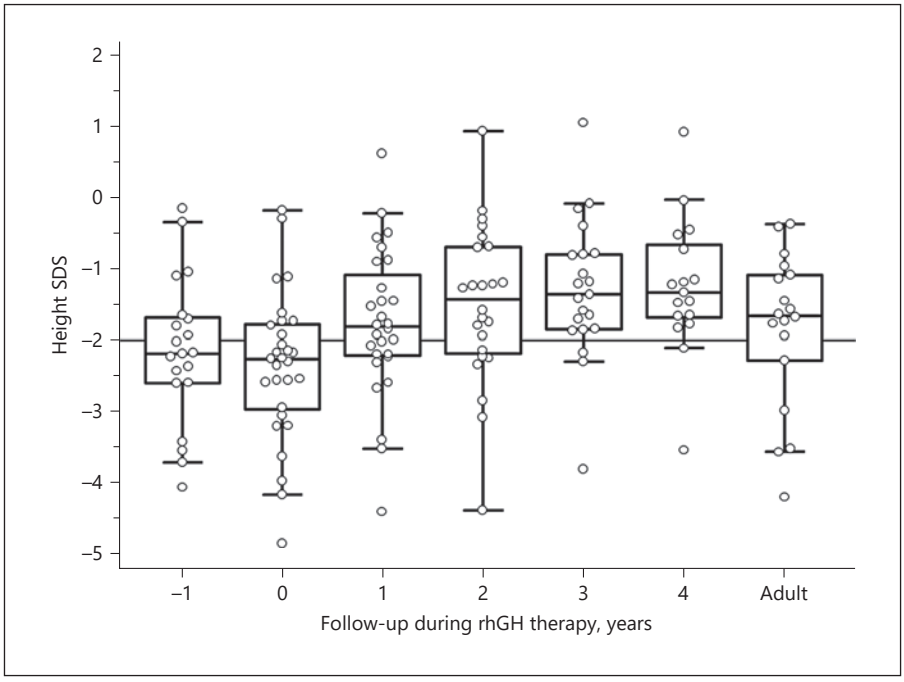

Fig. 1. Height SDS during follow-up in the rhGH-treated group with SHOX haploinsufficiency. SDS, standard deviation score; rhGH, recombinant human growth hormone.

observers based on the method of Greulich and Pyle [31], and the predicted adult height was calculated according to the Bayley-Pinneau method [32]. Target height was calculated by the mean parental height minus 6.5 cm for girls and plus 6.5 cm for boys [33].

In the rhGH-treated group, the initial dose was 50 μg/kg/day, and the subjects received a daily subcutaneous injection with a variety of commercially available rhGH products. The rhGH dose was adjusted according to the changes in weight to maintain 50 μg/kg/day. If IGF-1 SDS >2.0 was observed, rhGH dose was maintain stable until IGF-1 normalization. The rhGH treatment was suspended when the growth velocity was below 2 cm/year. Puberty modulators (PMs) were started in 4 girls due to central precocious puberty (age of thelarche ranged from 6.2 to 7.8 years). Additionally, the use of PMs was indicated also in patients with an adult height prediction below −2.0 SDS and/or a height SDS < −1.5 at the start of puberty. Three of the patients with precocious puberty were previously reported [34]. The therapy with PMs was discontinued at a bone age of 12 years and 13 years for girls and boys, respectively, and/or after a minimum duration treatment of 2 years.

Statistical Analysis

The qualitative (nominal) variables were evaluated with Fisher exact test and reported as percentages. The quantitative variables were evaluated with Mann-Whitney U statistics and expressed as median and interquartile range (p25–p75). The available clinical data were compared between treated and untreated groups and between rhGH alone and rhGH plus PMs. A p value less than 0.05 was used for statistical significance. All analyses were done with SigmaStat software (Systat Software, version 3.5, San Jose, CA, USA) and the figures with MedCalc Statistical Software (MedCalc Software Ltd., version 19.5.1, Ostend, Belgium) or using Growth Analyser 3.5 (Ed. Dutch Growth Foundation, PO Box 23068, 3001 KB, Rotterdam, The Netherlands).

Results

Baseline

In total, 47 patients with *SHOX* haploinsufficiency were selected: 37 patients with loss of function defects (nonsense point mutations or copy number variations affecting coding regions), 4 with missense mutations affecting *SHOX* homeodomain, and 6 with deletions restricted to regulatory regions (*SHOX* area). The baseline characteristics and the first year of follow-up of untreated and rhGH-treated patients are described in Table 1. Both groups had a similar chronological age at the start of follow-up, but patients who received rhGH therapy were shorter than who were not treated. The proportion of children with short stature (height SDS below −2.0) in the treated group (69%) was higher than in the untreated group (28%, $p = 0.008$) (Table 1). Despite a better baseline height SDS in the untreated group, both groups had similar adult height SDS predictions, reflecting an older bone age observed in the untreated group (median 12.0 years; interquartile range [IQR] of 9.1; 14.8) in comparison with the treated group (10.3 years [7.0; 11.0]; $p = 0.055$).

Short-Term Growth Response to rhGH

Twenty-nine children started rhGH treatment with the median dose of 49 μg/kg/day. In these children, rhGH promoted an increase in growth velocity that resulted in an improvement in height SDS during the first and sec-

Table 1. Baseline and first-year clinical data in untreated and rhGH-treated patients with *SHOX* haploinsufficiency

	N	Untreated group	*N*	rhGH-treated group	*p* value
Gender (female:male)	18	11:7	29	15:14	0.562
Genetic SHOX defect, %					
LoF mutation	15	83	22	76	0.719
Missense mutation	1	6	3	10	1.000
Deletion only in regulatory region	2	11	4	14	1.000
Index case,[a] %	18	50	29	79	0.055
ISS: DLW phenotype	18	12:6	29	17:12	0.759
Birth weight SDS	12	−0.3 (−1.2; 0.8)	29	−0.2 (−1.0; 0.6)	0.785
Birth length SDS	12	−1.4 (−2.3; −1.3)	26	−1.4 (−2.5; −0.6)	0.550
Born SGA, %	12	25[b]	27	41[c]	0.477
Height SDS of unaffected parent[d]	14	−1.1 (−1.6; 0.1)	31	−1.0 (−1.7; −0.5)	0.704
Height SDS of affected parent[d]	11	−2.5 (−3.2; −1.3)	25	−2.5 (−3.0; −1.5)	0.668
At baseline					
Chronological age, years	18	11.3 (9.4; 13.8)	29	10.1 (8.0; 11.7)	0.171
Height SDS	18	−1.7 (−2.3; −1.2)	29	−2.2 (−3.0; −1.8)	0.022
Short stature (height SDS < −2.0), %	18	28	29	69	0.008
BMI SDS	16	0.8 (0.3; 1.3)	27	0.5 (0.1; 1.7)	0.950
SH/H SDS	15	2.8 (1.9; 4.1)	27	3.3 (2.8; 4.0)	0.198
Arm span/height	10	0.97 (0.95; 0.99)	19	0.98 (0.96; 0.99)	0.477
Presence of Madelung deformity, %	18	33	29	41	0.759
Prepubertal, %	18	33	29	52	0.245
Bone age – chronological age, years	15	0.6 (−0.6; 1.4)	27	−0.7 (−1.4; 0.4)	0.109
Predicted adult height SDS	12	−2.0 (−2.7; −1.3)	23	−2.3 (−3.1; −1.6)	0.357
IGF-1 SDS	10	0.0 (−1.0; 1.1)	22	−0.2 (−0.8; 0.8)	0.730
rhGH dose, µg/kg/day		–		49.5 (49.5; 49.5)	–
PMs (in the first year)		–		10/29[e]	–
After 1 year of follow-up					
Height SDS	15	−1.7 (−2.2; −0.9)	28	−1.8 (−2.2; −1.1)	0.750
BMI SDS	15	0.6 (−0.2; 1.4)	27	0.4 (−0.2; 1.4)	0.979
SH/H SDS	10	3.0 (2.0; 3.7)	18	3.4 (2.1; 4.7)	0.581
Growth velocity, cm/year	14	6.2 (4.8; 7.2)	28	8.5 (6.8; 9.7)	0.004
Growth velocity SDS	14	−0.6 (−1.6; 1.0)	27	2.8 (1.5; −4.4)	<0.001
Height SDS changes (1st year – baseline)	15	−0.1 (−0.2; 0.1)	28	0.5 (0.3; 0.7)	<0.001
BMI SDS changes (1st year – baseline)	14	0.0 (−0.2; 0.2)	27	−0.1 (−0.5; 0.1)	0.201
SH/H SDS changes (1st year – baseline)	10	0.1 (−0.2; 0.5)	19	0.0 (−0.8; 0.6)	0.347
Bone age – chronological age, years	9	0.1 (−0.5; 0.8)	24	−0.6 (−1.2; 0.2)	0.163
Predicted adult height SDS change (1st year – baseline)	7	0.2 (−0.2; 0.5)	22	0.6 (0.2; 1.0)	0.063

Continuous variables are shown as median and IQR. SGA was defined as birth weight and/or birth length equal to or less than −2.0 SDS for sex and gestational age [29]. LoF, loss of function; ISS, idiopathic short stature; DLW, Leri-Weill dyschondrosteosis; SDS, standard deviation score; SGA, small for gestational age; BMI, body mass index; SH/H, sitting height-to-height ratio; rhGH, recombinant human growth hormone. [a] Percentage of index case in each group, in contrast with patients diagnosed by screening into the family. [b] Three born SGA for birth length. [c] One born SGA for birth weight and 10 for birth length. [d] For 3 patients, it was not possible to establish an inheritance pattern, and for 3 patients, parents' heights were not available. [e] Ten patients who used PMs started this therapy before or during the first year of rhGH treatment.

ond years of treatment (Fig. 1; Table 2). In the third and fourth years of treatment, the changes in the height SDS were similar. We did not observe changes in body proportions (sitting height-to-height ratio [SH:H]) or BMI during rhGH treatment. Bone age delay progressively decreased over the years. IGF-1 levels were normal at baseline in all patients and increased significantly during rhGH treatment (Table 2). We observed elevated IGF-1 levels (IGF-1 SDS >2) in 10 patients in the first year of rhGH therapy. The rhGH dose of these patients was not adjusted by weight and IGF-1 levels returned into the normal range in 6 of these patients during the follow-up.

The first-year growth response to rhGH treatment observed in 28 patients was compared with the 1-year follow-up of 15 patients without rhGH treatment. Treated patients had a growth velocity that was 2.3 cm/year greater than the untreated ones (Table 1), resulting in a difference of height SDS gain of 0.6 in favor of children who received rhGH therapy. Consequently, at the end of the first year of follow-up, both groups attained similar height SDS (Fig. 2; Table 1).

Puberty

At the start of puberty, patients with or without treatment had similar chronological age, bone age, and height SDS (Table 3). In the treated group, PMs were used in 60% of the girls (9/15, all GnRHa) and 29% of the boys (4/14, 2 GnRHa and 2 aromatase inhibitors). During the period from the onset of puberty to adult height, the untreated group lost height SDS. In contrast, the rhGH-treated group (alone or plus PMs) did not present a decrease in the height SDS. The median height gain from the start of puberty to adult height for girls was 23.5 cm when rhGH was used and 16.9 cm in patients followed without treatment ($p = 0.083$). For boys, the same favorable height gain was observed in treated (30.3 cm) patients in comparison with untreated ones (21.5 cm; $p = 0.036$). Rapid bone age maturation during puberty followed by early growth arrest and worsening of height SDS was documented in 8 patients followed without intervention; in 4 patients that received rhGH therapy alone; and two girls that received combine therapy (rhGH plus GnRHa) due to central precocious puberty (Fig. 3).

Adult Height and Long-Term Growth Response to rhGH

Adult height was available in 18 patients that received rhGH therapy and in 13 patients followed without treatment. The baseline characteristics and the auxological parameters of those patients who reached adult height are described in Table 3. At baseline, both groups had similar height SDS, although the treated group had a higher prevalence of short stature (61% in the treated group; 31% in the untreated group) and was slightly younger than the untreated one.

The treated group received a median rhGH dose of 49.5 μg/kg/day ([IQR] of [49.5; 49.5 μg/kg/day]), with a median duration of treatment of 4.6 (3.5; 6.1) years for rhGH and 2.4 (1.8; 3.9) years for PMs. During the follow-up, the untreated group had a negative median change in height SDS from baseline to adult height of −0.8, ranging from −1.5 to −0.2. In contrast, a positive change in height SDS

Table 2. Longitudinal follow-up in the rhGH-treated patients with *SHOX* haploinsufficiency

	Previous	Basal	1st year	2nd year	3rd year	4th year	End of rhGH	Adult
n	20	29	28	24	21	17	20	18
Sex (female:male)	9:11	15:14	14:14	12:12	9:12	6:11	11:9	12:6
Chronological age, years	9.8 (7.8; 11.0)	10.1 (8.0; 11.7)	11.6 (9.4; 12.7)	12.7 (9.7; 13.9)	13.1 (10.7; 14.8)	14.1 (11.6; 15.9)	14.8 (13.9; 16.4)	16.7 (15.9; 17.5)[a]
Bone age – chronological age, years	−0.9 (−1.6; −0.3)	−0.7 (−1.4; 0.4)	−0.6 (−1.2; 0.2)	−0.9 (−1.2; −0.1)	−0.4 (−1.1; 0.2)	0.0 (−0.8; 0.8)	–	–
Prepubertal, %	65	52	29	25	19	12	–	–
PMs	3	3	10	8	4	1	–	–
Growth velocity, cm/year	–	4.4 (3.7; 5.9)	8.5 (6.8; 9.7)[b]	6.6 (5.9; 7.7)[c]	5.8 (4.9; 7.4)	5.3 (3.9; 5.7)	–	–
Growth velocity SDS	–	−1.4 (−3.1; 0.2)	2.8 (1.5; 4.4)[b]	0.7 (−0.8; 2.3)[d]	0.5 (−0.9; 1.5)	0.2 (−1.3; 2.2)	–	–
Height SDS	−2.2 (−2.6; −1.7)	−2.2 (−3.0; −1.8)	−1.8 (−2.2; −1.1)[e]	−1.4 (−2.2; −0.7)	−1.3 (−1.8; −0.8)	−1.3 (−1.7; −0.7)	−1.3 (−1.7; 0.5)	−1.6 (−2.3; −1.1)
Height SDS changes	–	0.0 (−0.2; 0.1)	0.5 (0.2; −0.7)[b]	0.2 (0.0; 0.3)[d]	0.1 (−0.1; 0.3)	0.1 (−0.1; 0.1)	–	−0.3 (−0.5; 0.1)
BMI SDS	0.9 (−0.1; 1.7)	0.5 (0.1; 1.7)	0.4 (−0.2; 1.4)	0.5 (0.0; 1.7)	0.5 (0.0; 1.6)	0.6 (0.1; 1.7)	0.6 (0.1; 1.5)	–
SH/H SDS	3.4 (2.6; 4.0)	3.3 (2.8; 4.0)	3.4 (2.1; 4.7)	3.4 (2.6; 5.0)	3.8 (3.2; 4.6)	4.0 (3.4; 5.4)	4.2 (2.9; 5.5)	4.2 (3.0; 5.1)
IGF-1 SDS	–	−0.2 (−0.8; 0.8)	1.7 (0.6; 2.9)[b]	1.3 (0.4; 2.4)	1.6 (0.7; 2.2)	1.0 (0.5; 1.7)	–	–
Duration of rhGH treatment, years	–	–	–	–	–	–	4.3 (3.4; 5.4)	–
Duration of PM treatment, years	–	–	–	–	–	–	2.2 (1.9; 3.5)	–

Continuous variables are shown as median and interquartile range. SDS, standard deviation score; BMI, body mass index; SH/H, sitting height-to-height ratio; rhGH, recombinant human growth hormone; AH, adult height. Data in each column were compared with the data in the previous column, except to AH, in which height SDS change was calculated in relation to 4 years of treatment. The *p* values are below: [a] Comparison between AH and end of rhGH with a p value <0.001. [b] Comparison between 1st year and basal with a p value <0.001. [c] Comparison between 2nd year and 1st year with a p value <0.001. [e] Comparison between 2nd year and basal with a p value <0.05. [d] Comparison between 2nd year and 1st year with a p value <0.01.

Table 3. Clinical data in rhGH-treated and untreated patients with *SHOX* haploinsufficiency who reached adult height

	Untreated group (n = 13)	rhGH-treated group (n = 18)			p value	
		ALL (n = 18)	rhGH (n = 8)	rhGH + GnRHa (n = 10)	untreated versus treated group[a]	rhGH versus rhGH + GnRHa[b]
At baseline						
Sex (female:male)	9:4	12:6	4:4	8:2	1.000	0.321
Chronological age, years	12 (10.5; 14.2)	10.8 (7.8; 12.1)	9.6 (7.5; 11.8)	10.8 (9.1; 12.6)	0.043	0.307
Height SDS	−1.6 (−2.3; −1.2)	−2.2 (−2.9; −1.6)	−2.2 (−2.7; −1.9)	−2.0 (−3.6; −1.1)	0.180	0.756
Short stature (height SDS < −2.0), %	30.8	61.1	75	50	0.149	0.367
BMI SDS	0.9 (0.6; 1.3)	0.9 (0.0; 1.6)	0.4 (−0.4; 1.2)	1.4 (0.2; 1.8)	0.983	0.307
SH/H SDS	2.9 (2.6; −4.7)	3.4 (2.8; 5.0)	3.3 (2.7; 5.0)	3.3 (3.0; 4.8)	0.498	0.736
Presence of Madelung deformity, %	46.2	55.6	37.5	70	0.722	0.342
Prepubertal, %	15.4	44.4	75	20	0.129	0.054
Bone age – chronological age, years	1.2 (−0.2; 1.5)	−1.1 (−1.4; 0.1)	−1.3 (−1.6; −1.1)	−0.4 (−1.1; 1.3)	0.030	0.039
Predicted adult height SDS	−2.1 (−2.7; −1.6)	−2.4 (−3.3; −1.8)	−2.1 (−2.8; −1.7)	−3.0 (−3.7; −2.1)	0.276	0.316
At the start of puberty						
Chronological age, years	10.2 (9.8; 11.1)	11.0 (8.3; 12.0)	11.7 (10.6; 12.2)	9.4 (7.6; 11.7)	0.723	0.189
Height SDS	−1.2 (−1.6; −0.4)	−1.3 (−2.2; −0.7)	−1.2 (−2.2; −0.8)	−1.5 (−2.6; −0.2)	0.474	1.000
PM (GnRHa)	–	10/18	0/0	10/10	–	–
Duration of GnRHa treatment, years	–	2.4 (1.8; 3.9)	–	2.4 (1.8; 3.9)	–	–
Adult height						
rhGH dose, µg/kg/day	–	49.5 (49.5; 49.5)	49.5 (49.5; 49.5)	49.5 (49.5; 49.5)	–	<0.001
Duration of rhGH treatment, years	–	4.6 (3.5; 6.1)	5.3 (4.0; 6.9)	4.2 (3.5; 4.9)	–	0.351
Height SDS	−2.6 (−2.9; −1.9)	−1.6 (−2.3; −1.0)	−1.7 (−1.8; −1.5)	−1.4 (−3.0; −0.4)	0.034	0.625
Adult height SDS – PAH SDS	−0.1 (−0.4; −0.3)	0.7 (0.1; 1.2)	0.2 (−0.2; 0.4)	0.8 (0.6; 2.0)	0.007	0.052
Adult height SDS – baseline height SDS	−0.8 (−1.1; −0.4)	0.6 (0.2; 0.6)	0.5 (0.1; 1.0)	0.6 (0.2; 0.6)	<0.001	0.965
Adult height SDS – height SDS at start of puberty	−1.2 (−1.5; −0.6)	0.1 (−0.4; 0.6)	−0.2 (−0.7; 0.5)	0.2 (−0.2; 1.0)	0.002	0.259
Short stature (height SDS < −2.0), %	76.9	27.8	12.5	40	0.011	0.314
BMI	23.8 (20.7; 26.0)	23.2 (21.5; 26.7)	21.7 (19.9)	24.3 (22.9; 29.4)	0.783	0.056
SH/H SDS	3.2 (2.9; 4.5)	4.2 (3.0; 5.1)	5.0 (4.2; 5.2)	3.3 (2.8; 4.3)	0.427	0.075
SH/H SDS changes (adult height – baseline)	0.0 (−0.3; 0.4)	0.0 (−0.7; 0.9)	0.9 (0.1; 1.6)	−0.6 (−0.9; 0.1)	0.782	0.011
Presence of Madelung deformity, %	46.2	55.6	37.5	60	0.722	0.637

Continuous variables are shown as median and IQR. PMs, puberty modulators; rhGH, recombinant human growth hormone; SDS, standard deviation score; SGA, small for gestational age; BMI, body mass index; SH/H, sitting height to height; PAH, predicted adult height. [a] Untreated group versus rhGH-treated group. [b] rhGH alone versus rhGH plus PMs.

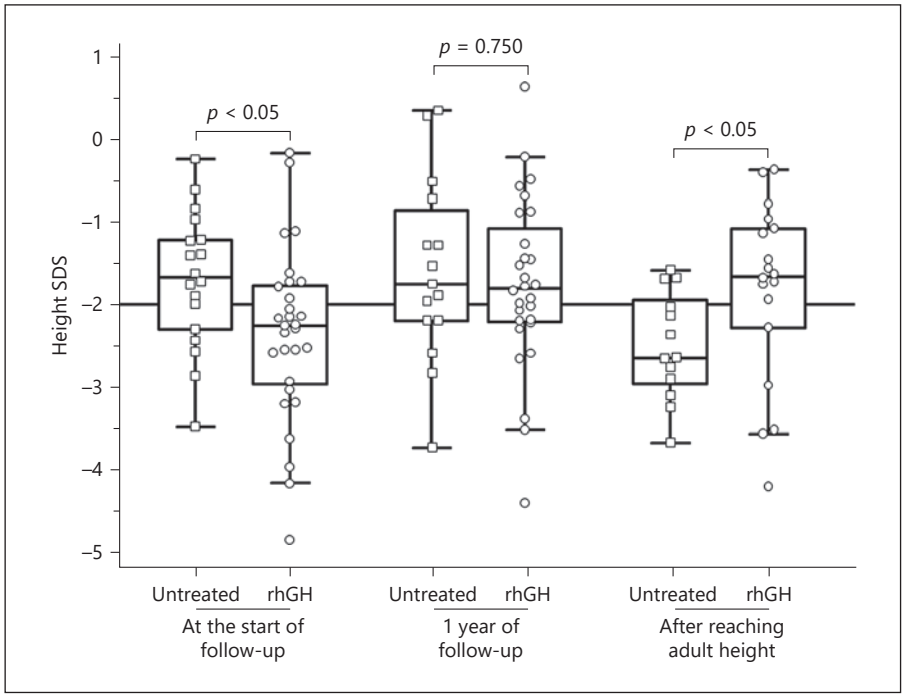

Fig. 2. Comparison between height SDS in untreated and rhGH-treated groups with *SHOX* haploinsufficiency. SDS, standard deviation score; rhGH, recombinant human growth hormone.

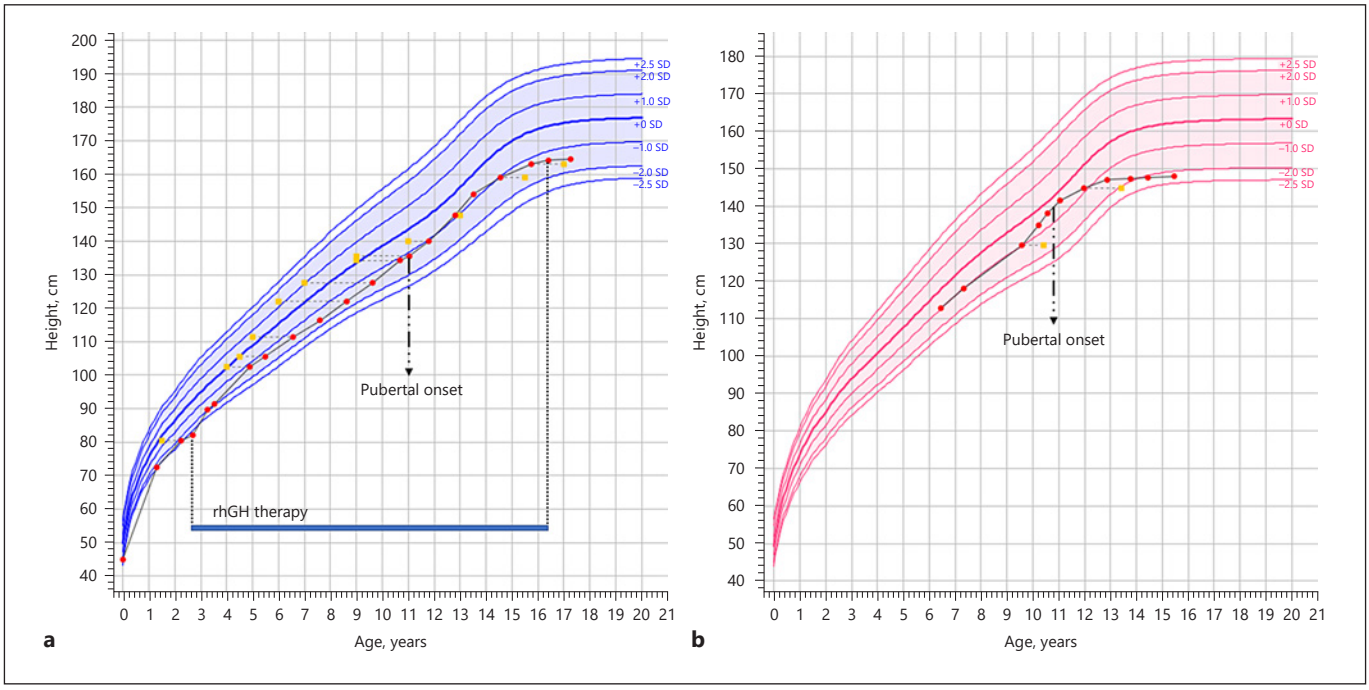

Fig. 3. Growth charts of patients with *SHOX* haploinsufficiency: one boy who used rhGH treatment (**a**) and one girl who refused rhGH treatment (**b**). Heights are plotted against age as red solid circles. Bone ages are plotted as yellow solid circles (assessment by the Greulich-Pyle method [31]). An arrow indicates the pubertal onset. The growth chart was drawn using Growth Analyser 3.5 (Ed. Dutch Growth Foundation, PO Box 23068, 3001 KB, Rotterdam, The Netherlands). SD, standard deviation score; rhGH, recombinant human growth hormone.

Table 4. Studies on adult height after rhGH therapy with or without PMs in patients with *SHOX* haploinsufficiency

Study	Study protocol	Patients,[a] *n*	rhGH treatment			Duration of PM treatment, years (Patients, *n*)	Adult height SDS[b]	Δ Height SDS[c]
			CA at the start, years	dose, μg/kg/day	duration, years			
Ogata et al. [19]	Retrospective	1	6.0	23.8	8.0	3.0 (*n* = 1)[d]	−4.1	−0.1
Munns et al. [20]	Retrospective	1	13.6	44.4	2.0	–	−3.0	1.1
Blum et al. [21]	Retrospective	14	10.3±3.0	35.7±15.7	4.7±2.3	–	−2.2±0.8	1.1
Scalco et al. [15]	Retrospective	5[e]	11.8±2.1	50±NA	3.8±1.1	2.8±1.8 (*n* = 5)[d]	−1.7±1.7	0.6±0.4
Salmon-Musial et al. [11]	Retrospective	5	9.6±2.7	NA	5.2±2.2	NA (*n* = 1)[d]	−2.9±0.8	0.6±1.0
Blum et al. [22]	Prospective clinical trial	28	9.2±2.4	52.9±2.9	6.0±2.0	–	−1.95±1.32	1.25±0.85
Benabbad et al. [23]	Prospective observational	90	11.0±2.4	47.1±14.3	4.4±2.3	–	−2.18±NA	0.83±NA
Present study	Retrospective	18	10.1±2.8	49.5±0.0	5.0±2.7	2.9±1.4 (*n* = 10)[d]	−1.8±1.1	0.4±0.7
All	–	157[f]						

Continuous variables are shown as mean±standard deviation. CA, chronological age; PMs, puberty modulators; NA, not available or not applicable; rhGH, recombinant human growth hormone; SDS, standard deviation score; AH, adult height. [a] Number of rhGH-treated patients. [b] Adult height SDS calculated according to local growth charts or CDC growth charts. [c] Change in height SDS from baseline to adult height. [d] Number of patients who used rhGH treatment plus puberty modulators. [e] Not included in the final analysis since 5 patients were included in both studies. [f] Total number of rhGH-treated patients evaluated in the studies.

from baseline to adult height was observed in patients who were treated with rhGH (median of 0.6; range from −1.4 to 1.6) (Fig. 2). In the treated group, adult height SDS improved in relation to the initial adult height prediction, whereas this was not observed in the untreated group.

At the end of the growth period, the treated group was 1 SD (~6.3 cm) taller than the untreated group. The prevalence of short stature (height SDS < −2.0) from baseline to adult height increased in the untreated group (from 31% to 77%), while it decreased in the treated group (from 61% to 28%). When comparing adult height between patients and their parents with *SHOX* haploinsufficiency that share the same molecular defect, the untreated group had a similar adult height (−2.6 [IQR −2.9; −1.9]) vs. −2.4 [IQR −2.5; −1.1], respectively, $p = 0.182$) and the treated one was taller than their parents (−1.6 [IQR −2.3; −1.0] vs. −2.2 [IQR −2.8; −1.4], respectively, although it did not reach statistical significance, $p = 0.393$). We did not observe differences in body proportions, BMI, and in the presence of Madelung deformity at adult age between patients with *SHOX* haploinsufficiency treated or not with rhGH.

rhGH Alone versus rhGH plus PMs

The majority of the patients in the group that used rhGH plus PMs (GnRHa or AI) were pubertal when they started the rhGH treatment with a slightly worse adult height prediction in comparison with patients who used rhGH alone (Table 3). None of the two boys treated with AI achieved adult height at the end of the study. At the moment of PM withdrawal, the girls had a median chronological age of 13.1 years (IQR 11.7; 14.4), bone age delay of −1.3 (IQR −1.6; 0.1), and height SDS of −1.4 (IQR −2.9; −0.2), while the boys had a median chronological age of 14.5 years (IQR 14.1; 15.3), bone age delay of −1.3 (IQR −2.3; −0.3), and height SDS of −1.6 (IQR −2.1; −1.0). Nevertheless, the adult height achieved in patients treated or not with GnRHa was similar, with a slightly better height SDS change in relation to adult height prediction obtained at the baseline in favor of patients who used PMs (0.2 vs. 0.8, respectively; $p = 0.052$). It is noteworthy that patients treated with GnRHa presented attenuation of body disproportion in comparison to patients treated with rhGH alone (SH/H SDS median changes from baseline to adulthood for patients treated with combined therapy or rhGH alone were −0.6 and 0.9, respectively; $p = 0.011$; Table 3). Adult height was reached for 3 of the 4 girls with *SHOX* haploinsufficiency and central precocious puberty after rhGH plus GnRHa treatment (median treatment duration of 4.9 and 3.9 years, respectively). All of them reached an adult height within the reference range (height SDS of −0.4, −1.1, and −1.6).

Discussion

The use of rhGH in patients with isolated *SHOX* haploinsufficiency is well accepted due to the similar pathophysiology to the Turner syndrome [1] and to the few

studies that assessed the short- [5, 6, 23, 35, 36] and long-term [11, 15, 19–23] effectiveness of this treatment (Table 4). However, there is a paucity of studies that compared the adult height of patients with or without intervention. In this context, we reported the adult height of 31 patients with *SHOX* haploinsufficiency, 13 followed without growth promoting therapy, and 18 patients that received rhGH with or without associated GnRHa. This longitudinal retrospective long-term study emphasizes the real-world effectiveness of rhGH treatment in children with *SHOX* haploinsufficiency.

Longitudinal follow-up studies until adult height in patients with isolated *SHOX* haploinsufficiency, particularly in untreated ones, are limited. We demonstrated that patients with *SHOX* haploinsufficiency followed without therapeutic intervention showed a reduction in height SDS from childhood to adulthood (with a median decrease of 0.8) and reached an adult height 1.0 SDS (~6.3 cm) lower than patients who received rhGH treatment (Fig. 2; Table 3). In contrast, patients who were treated with rhGH alone or plus GnRHa reached an adult height SDS greater than predicted (a difference that corresponds to 4.2 cm) and observed at the first evaluation (with a median increase in height SDS of 0.6). However, the total height SDS change observed in our patients that received rhGH was lower than described in previous studies (Table 4). It might be explained by the fact that several of our children started treatment just after pubertal onset and by the high prevalence of LoF and missense mutation in *SHOX* gene in our cohort. The regulatory region deletion is a well-known alteration associated with a better response to rhGH therapy [35].

We observed an increase in the prevalence of short stature during the follow-up in the untreated group, from 31% at the first evaluation to 77% at the end of growth. The opposite was observed in patients that received rhGH treatment, in whom the prevalence of short stature decreased from 61% at the start of rhGH treatment to 28% at the end of growth. The recommendation to treat children with *SHOX* haploinsufficiency that have height within the lower normal range has not yet been established. Our results support the ideas that the diagnosis of a *SHOX* defects could make an important contribution in the decision-making process. For this reason, these children should be closely followed due to the probability of losing height potential during puberty.

The first-year growth response explained part of the total height improvement observed in patients who received rhGH. The untreated group had a median loss of height SDS of −0.1 in the first year of follow-up and maintained a growth velocity in the lower range of normality (Table 1), as previously described by other longitudinal follow-up studies [6]. On the other hand, patients who used rhGH had a median gain in height SDS of 0.5 (Fig. 1) and a significantly increase in growth velocity SDS from −1.4 to 2.8, which continued in the upper range of normality in the following years (Table 2). These results confirm the good short-term response to rhGH treatment in these patients, as the gain in height SDS in the first year varied from 0.43 to 0.7 [6, 23, 35, 36], being greater in prepubertal children [6].

Longitudinal follow-up studies in patients with *SHOX* haploinsufficiency had described a preserved timing of onset and progression of puberty [22], but a reduced growth spurt with a worsening of height SDS [13–15] due to the advance in bone age during this phase [14, 21, 22, 36]. This observation can be due to an additional effect of estrogens over a reduced SHOX action in the hypertrophic chondrocytes zone in growth plate, leading to precocious fusion during puberty and the loss of growth potential observed in these patients [4, 37]. Our study additionally characterizes growth throughout puberty with or without intervention. The untreated group had an onset of puberty at the normal age, but a total height gain during puberty (16.9 cm in girls and 21.5 cm in the boys) smaller than expected when compared with children with normal growth (average of 25 cm in girls and 28 cm in boys) [26, 38]. The height SDS in the treated group was practically sustained from the onset of puberty to adulthood (Table 3), with a slight increase in the group with combined therapy, whereas the untreated group lost 1.2 height SDS. This finding supports the fact that rhGH treatment with or without PMs could avoid the height SDS loss that occurs during this phase in untreated patients. It is noteworthy that four of the girls in our cohort had central precocious puberty (all of them treated with rhGH and GnRHa); however, we cannot exclude the possibility of a random association [34].

The advance in bone age during puberty in patients with *SHOX* haploinsufficiency could suggest a better responsiveness in those treated with rhGH plus PMs. There are few reports on use of PMs in *SHOX* haploinsufficiency [11, 15, 19] and none compares patients treated with rhGH alone with combined therapy. Our results showed similar adult heights for patients treated with rhGH alone or rhGH plus PMs, although the latter group started rhGH therapy at a slightly older age, more frequently after puberty onset, and had a better height SDS change in relation to predicted adult height obtained at the baseline (Table 3). Interestingly, we observed an improvement in

body proportions in children who were treated with combined therapy, although this observation is limited by the small number of patients in each group. In patients who do not have the opportunity to start treatment in the prepuberal age or who have a worse adult height prediction, the use of combination therapy can be a strategy to improve adult height.

Few studies analyzed the adult height of patients with isolated *SHOX* haploinsufficiency and the impact of rhGH therapy. Although limited by its retrospective design and by a relatively small number of patients that reached adult height, our study has provided a deeper insight into the natural growth patterns of children with *SHOX* defects and into the benefits of rhGH treatment with or without PMs to improve adult height. Those children with heights within the reference range need careful monitoring as they often show a negative change in height SDS during puberty and treatment with rhGH can prevent this loss in height potential. Children with late diagnosis or poor adult height predictions may benefit from combined treatment with rhGH plus PMs.

Statement of Ethics

This study protocol was approved by the Hospital das Clinicas Ethics Committee (approval number CAAE-37868114.3.0000.0068). Parental consent for molecular analysis and clinical information assessment was obtained after full explanation of the purpose of this study. This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Conflict of Interest Statement

A.A.L.J. has received speaker fees from Novo Nordisk and Pfizer; has independent research grant from BioMarin; and has received consulting fees from Novo Nordisk. The other authors declare that they have no competing financial interest to declare.

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Author Contributions

N.C.B.D. made substantial contributions to acquisition and analysis of data and helped drafting the manuscript. M.F.A.F., G.A.V., N.L.M.A., R.C.R., V.B., and R.C.S. made substantial contributions to acquisition of data and revised it critically for important intellectual content. I.J.P.A. and B.B.M. made substantial contributions to the conception of the work and revised it critically for important intellectual content. A.A.L.J. made substantial contributions to the conception of the work, acquisition, analysis, and interpretation of data and helped drafting the manuscript. All authors approved the final submitted version and agreed to be accountable for the accuracy and integrity of the work.

Data Availability Statement

The datasets generated and/or analyzed during this study are not publicly available but are available from the corresponding author upon reasonable request.

References

1 Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, et al. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat Genet. 1997 May;16(1):54–63. https://doi.org/10.1038/ng0597-54.

2 Ellison JW, Wardak Z, Young MF, Gehron Robey P, Laig-Webster M, Chiong W. PHOG, a candidate gene for involvement in the short stature of Turner syndrome. Hum Mol Genet. 1997 Aug;6(8):1341–7.

3 Marchini A, Ogata T, Rappold GA. A track record on SHOX: from basic research to complex models and therapy. Endocr Rev. 2016;37(4):417–48.

4 Marchini A, Marttila T, Winter A, Caldeira S, Malanchi I, Blaschke RJ, et al. The short stature homeodomain protein SHOX induces cellular growth arrest and apoptosis and is expressed in human growth plate chondrocytes. J Biol Chem. 2004 Aug;279(35):37103–14. https://doi.org/10.1074/jbc.M307006200.

5 Binder G, Schwarze CP, Ranke MB. Identification of short stature caused by SHOX defects and therapeutic effect of recombinant human growth hormone. J Clin Endocrinol Metab. 2000 Jan;85(1):245–9.

6 Blum WF, Crowe BJ, Quigley CA, Jung H, Cao D, Ross JL, et al. Growth hormone is effective in treatment of short stature associated with short stature homeobox-containing gene deficiency: two-year results of a randomized, controlled, multicenter trial. J Clin Endocrinol Metab. 2007 Jan;92(1):219–28. https://doi.org/10.1210/jc.2006-1409.

7 Jorge AAL, Souza SC, Nishi MY, Billerbeck AE, Libório DCC, Kim CA, et al. SHOX mutations in idiopathic short stature and Leri-Weill dyschondrosteosis: frequency and phenotypic variability. Clin Endocrinol. 2007 Oct;66(1):130–5. https://doi.org/10.1111/j.1365-2265.2006.02698.x.

8 Binder G, Renz A, Martinez A, Keselman A, Hesse V, Riedl SW, et al. SHOX haploinsufficiency and Leri-Weill dyschondrosteosis: prevalence and growth failure in relation to mutation, sex, and degree of wrist deformity. J Clin Endocrinol Metab. 2004 Sep;89(9):4403–8. https://doi.org/10.1210/jc.2004-0591.

9 Belin V, Cusin V, Viot G, Girlich D, Toutain A, Moncla A, et al. SHOX mutations in dyschondrosteosis (Leri-Weill syndrome). Nat Genet. 1998 May;19(1):67–9. https://doi.org/10.1038/ng0198-67.

10 Leri A, Weill J. Une affection congenitale et symetrique du developpement osseux: la dyschondrosteose. Bull Mem Soc Med Hop. 1929 [cited 2021 Aug 19];35:1491–4.
11 Salmon-Musial AS, Rosilio M, David M, Huber C, Pichot E, Cormier-Daire V, et al. Clinical and radiological characteristics of 22 children with SHOX anomalies and familial short stature suggestive of Léri-Weill dyschondrosteosis. Horm Res Paediatr. 2011;76(3):178–85. https://doi.org/10.1159/000329359.
12 Urakami T, Hasegawa M, Morimoto S, Saitoh H, Mugishima H. Effect of growth hormone therapy in two sisters with SHOX haploinsufficiency. Pediatr Int. 2009 Aug;51(4):574–6.
13 Kosho T, Muroya K, Nagai T, Fujimoto M, Yokoya S, Sakamoto H, et al. Skeletal features and growth patterns in 14 patients with haploinsufficiency of SHOX: implications for the development of Turner syndrome. J Clin Endocrinol Metab. 1999 Dec;84(12):4613–21. https://doi.org/10.1210/jcem.84.12.6289.
14 Fukami M, Matsuo N, Hasegawa T, Sato S, Ogata T. Longitudinal auxological study in a female with SHOX (short stature homeobox containing gene) haploinsufficiency and normal ovarian function. Eur J Endocrinol. 2003 Oct;149(4):337–41.
15 Scalco RC, Melo SSJ, Pugliese-Pires PN, Funari MFA, Nishi MY, Arnhold IJP, et al. Effectiveness of the combined recombinant human growth hormone and gonadotropin-releasing hormone analog therapy in pubertal patients with short stature due to SHOX deficiency. J Clin Endocrinol Metab. 2010 Jan; 95(1): 328–32. https: //doi.org/10.1210/jc.2009-1577.
16 Ross JL, Scott C, Marttila P, Kowal K, Nass A, Papenhausen P, et al. Phenotypes associated with SHOX deficiency. J Clin Endocrinol Metab. 2001 Dec;86(12):5674–80. https://doi.org/10.1210/jcem.86.12.8125.
17 Del Pino M, Aza-Carmona M, Medino-Martín D, Gomez A, Heath KE, Fano V, et al. SHOX deficiency in argentinean cohort: long-term auxological follow-up and a family's new mutation. J Pediatr Genet. 2019 Sep; 8(3):123–32. https://doi.org/10.1055/s-0039-1691788.
18 Malaquias AC, Scalco RC, Fontenele EGP, Costalonga EF, Baldin AD, Braz AF, 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):449–56. https: //doi.org/10.1159/000355411.
19 Ogata T, Onigata K, Hotsubo T, Matsuo N, Rappold G. Growth hormone and gonadotropin-releasing hormone analog therapy in haploinsufficiency of SHOX. Endocr J. 2001 Jun;48(3):317–22.
20 Munns CFJ, Berry M, Vickers D, Rappold GA, Hyland VJ, Glass IA, et al. Effect of 24 months of recombinant growth hormone on height and body proportions in SHOX haploinsufficiency. J Pediatr Endocrinol Metab. 2003 Jan;16(7):997–1004. https://doi.org/10.1515/JPEM.2003.16.7.997.
21 Blum WF, Cao D, Hesse V, Fricke-Otto S, Ross JL, Jones C, et al. Height gains in response to growth hormone treatment to final height are similar in patients with SHOX deficiency and Turner syndrome. Horm Res. 2009; 71(3): 167–72. https: //doi.org/10.1159/000197874.
22 Blum WF, Ross JL, Zimmermann AG, Quigley CA, Child CJ, Kalifa G, et al. GH treatment to final height produces similar height gains in patients with SHOX deficiency and turner syndrome: results of a multicenter trial. J Clin Endocrinol Metab. 2013;98(8):E1383-92. https://doi.org/10.1210/jc.2013-1222.
23 Benabbad I, Rosilio M, Child CJ, Carel JC, Ross JL, Deal CL, et al. Safety outcomes and near-adult height gain of growth hormone-treated children with SHOX deficiency: data from an observational study and a clinical trial. Horm Res Paediatr. 2017;87(1):42–50. https://doi.org/10.1159/000452973.
24 Funari MFA, de Barros JS, Santana LS, Lerario AM, Freire BL, Homma TK, et al. Evaluation of SHOX defects in the era of next-generation sequencing. Clin Genet. 2019 Sep;96(3):261–5. https://doi.org/10.1111/cge.13587.
25 Funari MFA, Jorge AA, Souza SC, Billerbeck AE, Arnhold IJ, Mendonca BB, et al. Usefulness of MLPA in the detection of SHOX deletions. Eur J Med Genet. 2010;53(5):234–8. https://doi.org/10.1016/j.ejmg.2010.06.001.
26 Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969 Jun;44(235):291–303.
27 Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, et al. 2000 CDC growth charts for the United States: methods and development. Vital Health Stat. 2002 May;11246(246):1–190.
28 Gerver WJM, Bruin R. Paediatric morphometrics: a reference manual. 2001. Available from: https://cris.maastrichtuniversity.nl/en/publications/paediatric-morphometrics-a-reference-manual.
29 Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks. J Pediatr. 1969 Jun;74(6):901–10. https: //doi.org/10.1016/S0022-3476(69)80224-6.
30 Tanner JM, Whitehouse RH, Takaishi M. Standards from birth to maturity for height, weight, height velocity, and weight velocity: british children, 1965. II. Arch Dis Child. 1966 Dec;41(220):613–35.
31 Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. Am J Med Sci. 1959 Sep;238(3):393.
32 Bayley N, Pinneau SR. Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standards. J Pediatr. 1952 Apr;40(4):423–41.
33 Tanner JM, Goldstein H, Whitehouse RH. Standards for children's height at age 2 to 9 years allowing for height of parents. Arch Dis Child. 1970 Dec;45(244):819.
34 Canton APM, Krepischi ACV, Montenegro LR, Costa S, Rosenberg C, Steunou V, et al. Insights from the genetic characterization of central precocious puberty associated with multiple anomalies. Hum Reprod. 2021 Jan; 36(2):506–18. https://doi.org/10.1093/humrep/deaa306.
35 Donze SH, Meijer CR, Kant SG, Zandwijken GR, van der Hout AH, van Spaendonk RM, et al. The growth response to GH treatment is greater in patients with SHOX enhancer deletions compared to SHOX defects. Eur J Endocrinol. 2015 Nov;173(5):611–21. https://doi.org/10.1530/EJE-15-0451.
36 Iughetti L, Vannelli S, Street ME, Pirazzoli P, Bertelloni S, Radetti G, et al. Impaired GH secretion in patients with SHOX deficiency and efficacy of recombinant human GH therapy. Horm Res Paediatr. 2012;78(5–6):279–87. https://doi.org/10.1159/000345354.
37 Weise M, De-Levi S, Barnes KM, Gafni RI, Abad V, Baron J. Effects of estrogen on growth plate senescence and epiphyseal fusion. Proc Natl Acad Sci U S A. 2001 Jun; 98(12):6871–6.
38 Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970 Feb;45(239):13–23.

3.4 CAPÍTULO 3: ADULT HEIGHT IN 299 PATIENTS WITH TURNER SYNDROME WITH OR WITHOUT GROWTH HORMONE THERAPY: RESULTS AND LITERATURE REVIEW

Dantas NCB, Braz AF, Malaquias A, Lemos-Marini S, Arnhold IJP, Silveira ER, Antonini SR, Guerra-Junior G, Mendonca B, Jorge A, Scalco RC. Adult Height in 299 Patients with Turner Syndrome with or without Growth Hormone Therapy: Results and Literature Review. Horm Res Paediatr. 2021;94(1-2):63-70. doi: 10.1159/000516869.

Este estudo é produto de um esforço multicêntrico (USP-SP, USP-RP, UNICAMP) onde reunimos uma grande coorte de pacientes acompanhados por Síndrome de Turner que atingiram altura adulta. Estudos prévios que analisam a efetividade do tratamento com hormônio do crescimento no ganho de altura adulta na ST usam coortes históricas ou previsão de altura adulta para avaliar resposta ao tratamento. Neste estudo, analisamos resposta ao tratamento com hormônio do crescimento das pacientes com Síndrome de Turner comparando um grupo não tratado com um grupo tratado que atingiram altura adulta usando evidências de mundo real.

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Adult Height in 299 Patients with Turner Syndrome with or without Growth Hormone Therapy: Results and Literature Review

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Keywords
Adult height · Turner syndrome · Growth hormone therapy

Abstract
Context: Treatment with growth hormone (GH) is considered effective in improving adult height (AH) in Turner syndrome (TS). However, there are few studies comparing AH between treated patients and a concurrent untreated group. ***Objective:*** To assess the efficacy of GH treatment in improving AH in TS and to review previous published studies with treated and untreated groups. ***Participants and Methods:*** We retrospectively analyzed clinical data and AH of a large cohort of GH-treated (n = 168) and untreated (n = 131) patients with TS. Data are shown as median and interquartile range (IQR). We assessed pretreatment variables related with AH and compared our results with 16 studies that also included an untreated group. ***Results:*** The GH-treated group was 6.2 cm taller than the untreated group (AH = 149 cm [IQR 144.5–152.5 cm] vs. 142.8 cm [IQR 139–148 cm], $p < 0.001$) after 4.9 years of GH treatment with a dose of 0.35 mg/kg/week. AH SDS corrected for target height (TH) was 7.2 cm higher in GH-treated patients. AH SDS ≥–2 was more frequent in GH-treated patients (43%) than in untreated patients (16%, $p < 0.001$). AH SDS was also more frequently within the TH range in the GH-treated group (52%) than in the untreated group (15%, $p < 0.001$). Height SDS at start of GH therapy and TH SDS were positively correlated with AH ($p < 0.001$; $R^2 = 0.375$). Considering the current result together with previous similar publications, a mean AH gain of 5.7

Alexander Jorge and Renata C. Scalco contributed equally to the manuscript.

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cm was observed in GH-treated (n = 696) versus untreated (n = 633) patients. ***Conclusions:*** Our study strengthens the evidence for efficacy of GH therapy in patients with TS from different populations.

Introduction

Turner syndrome (TS) is a chromosomal disorder characterized by complete or partial loss of the second sex chromosome [1] and affects 1 in every 2,500 females [2]. Short stature is the most common clinical manifestation, present in 98% of patients [3]. Although the mechanisms of growth failure in TS are not completely understood, the main cause appears to be a dysregulated endochondral ossification secondary to the haploinsufficiency of the short stature homeobox (*SHOX*) gene, which regulates chondrocyte differentiation and maturation [4]. Partial intrauterine growth restriction associated with diminished prepubertal growth and loss of pubertal growth spurt [5] result in an average adult height 20 cm shorter than general female population in untreated patients [6].

Since the 1980s, when recombinant human growth hormone (rhGH) became available [7], many clinical trials showed a mean height gain of 5–8 cm in patients with TS after rhGH treatment, leading to the approval of this treatment in many countries. However, most studies did not have an untreated/placebo control group, basing these results on baseline-predicted adult height or on the comparison with historical controls [3]. After the approval of rhGH treatment, there are also ethical restrictions on having an untreated group for comparison.

In the present study, we had the opportunity to compare the adult height results of a large cohort of rhGH-treated patients (n = 168) to the data from an untreated cohort (n = 131). We also compared observed adult height to predicted adult height based on bone age before the start of rhGH therapy. Finally, we analyzed our data and reviewed previous studies that compared adult height in patients with TS with and without rhGH therapy to evaluate total height gain improvement and factors that can modulate the response to rhGH therapy in TS.

Subjects and Methods

Subjects

This retrospective study included 299 patients with TS followed in the pediatric and endocrinology outpatient clinics of 3 university hospitals based in the state of Sao Paulo, Brazil: 207 from Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo (HC-FMUSP), 78 from Hospital das Clinicas da Faculdade de Ciencias Medicas da Universidade Estadual de Campinas (HC-FCM-UNICAMP), and 14 from Hospital das Clinicas da Faculdade de Medicina de Ribeirao Preto da Universidade de Sao Paulo (HC-FMRP-USP).

Among these patients, 168 were treated with rhGH (154 from HC-FMUSP and 14 from HC-FMRP-USP) and 131 were not treated because they had a late diagnosis, precluding rhGH therapy, or because they were diagnosed before routine use of rhGH for TS (53 from HC-FMUSP and 78 from HC-FCM-UNICAMP). The inclusion criteria were as follows: (1) diagnosis by peripheral blood karyotype, (2) attainment of adult height, which was defined as growth velocity equal or inferior to 0.5 cm/year, and (3) rhGH therapy duration of at least 2 years (for the treated group). The exclusion criteria were (1) the presence of severe diseases that could affect adult height and (2) irregular use of rhGH (Fig. 1).

The study protocol was approved by the Research Ethics Committees of HC-FMUSP, HC-FCM-UNICAMP, and HC-FMRP-USP. Written informed consent was obtained from all patients.

Methods

Karyotype was analyzed from peripheral blood leukocytes by standard methods in all patients and classified for analysis as 45,X, 45,X/46,XX, presence of Y chromosome or Y-chromosome markers, and others (including chromosomal abnormalities such as isochromosome Xq and ring X chromosome). Height was measured on a stadiometer graduated in millimeters, weight was measured on a digital scale, and pubertal stage was assessed according to Tanner stages [8]. Height, weight, and BMI SDS were calculated based on the Centers for Disease Control and Prevention (CDC) growth charts [9]. The date of onset of puberty was defined by the first visit on which spontaneous breast development was observed (Tanner stage greater than B1) or by the beginning of estrogen replacement. Bone age was assessed using the Greulich-Pyle method [10], and predicted adult height was calculated according to the Bayley-Pinneau method [11]. Target height (TH) was calculated by the mean parental height minus 6.5 cm [12].

Statistical Analysis

Continuous variables were expressed as median and interquartile range (IQR) (p25–p75). Qualitative (nominal) variables were reported as percentages. To compare treated and untreated groups, we used the Mann-Whitney rank sum test for continuous variables and the z test for proportions of nominal variables. To assess whether clinical variants at the beginning of the treatment had independent prognostic significance for adult height outcome, we performed linear regression analysis with pretreatment data followed by multiple regression analysis including variables that reached statistical significance in the univariate analysis. A p value <0.05 was used for statistical significance. All analyses were done with SigmaStat software (RRID:SCR_010285, version 3.5; Systat Software, San Jose, CA) and the figures with MedCalc Statistical Software (RRID:SCR_015044, version 19.5.1; MedCalc Software Ltd., Ostend, Belgium).

Literature Review

We searched MEDLINE, EMBASE, and SCIELO databases until May 31, 2020, for the terms "Turner syndrome" or "Turner's syndrome" or "Ullrich-Turner syndrome" and "adult height" or "final height" and "growth hormone" or "somatotropin" or "soma-

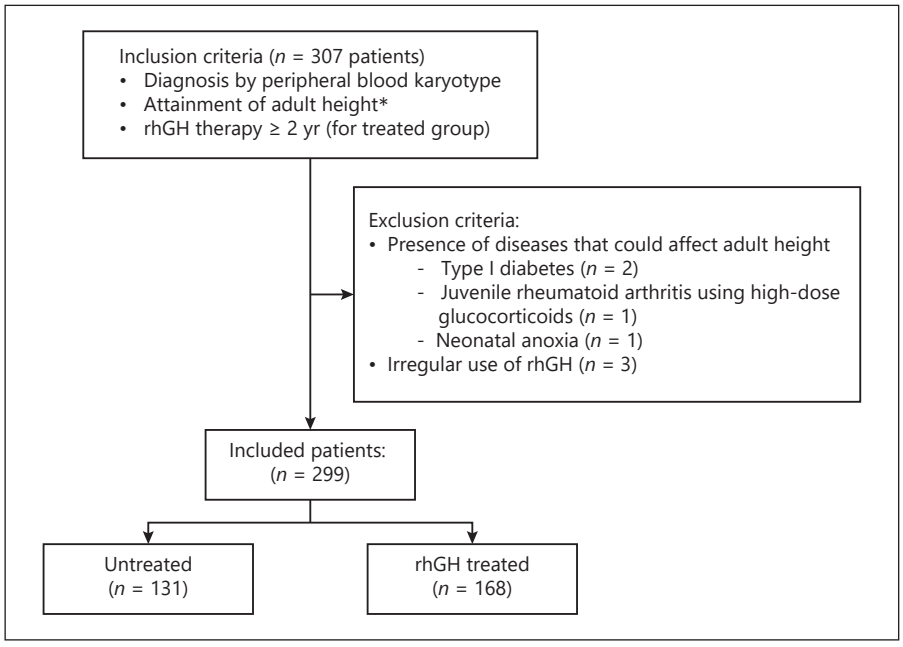

Fig. 1. Flow diagram with inclusion and exclusion criteria for patient selection. *Defined as growth velocity equal or inferior to 0.5 cm/year. rhGH, recombinant human growth hormone.

tropin." We also searched the reference lists of the selected original articles and reviews for additional references. We selected studies published in English, which compared rhGH-treated and untreated groups, excluding studies (or groups within studies) in which oxandrolone was used. We also excluded studies (or groups within studies) in which estrogens were used before 11 years old as a possible adjunctive treatment for height. Two authors independently searched for articles, selected relevant studies, and extracted data. Disagreements were resolved by a third author.

Results

Studied Cohort

Clinical Data in rhGH-Treated and Untreated Patients with TS

The characteristics of rhGH-treated and untreated patients with TS are depicted in Table 1. As a group, untreated patients were older at first evaluation, had a greater frequency of 45,X/46,XX karyotype, higher TH, and older age at the start of puberty (spontaneous and induced) than rhGH-treated patients. Conversely, rhGH-treated patients had a higher frequency of 45,X karyotype and of oxandrolone use. The frequency of patients with karyotypes with Y chromosome or Y-chromosome markers was not significantly different between the groups, and adult height in these patients was not significantly different from adult height in patients with other karyotypes in rhGH-treated (151.8 vs. 149 cm, $p = 0.727$) and untreated (145.8 vs. 142.6 cm, $p = 0.116$) groups. The median difference in the year of birth between the 2 groups was of a decade, a finding that can be explained by the fact that a subgroup of untreated patients was diagnosed before routine use of rhGH for TS. Bone age was not available for most untreated patients since 77% of them had already attained adult height at first evaluation.

At the start of rhGH therapy, 89% of patients in the treated group were prepubertal, with a median age of 11 years old. The median dose used was 0.35 mg/kg/week, and the duration of rhGH treatment was 4.9 years (IQR: 3.5, 7.8). The first-year growth velocity (available in 133/168 rhGH-treated patients) was 7.1 cm/year (IQR: 6.0, 8.3), resulting in a height SDS increase in the first year of treatment of 0.44 (IQR 0.2, 0.65).

Adult height was significantly higher in the rhGH-treated group than in the untreated group (149 vs. 142.8 cm, $p < 0.001$; Fig. 2a; Table 1), with a median gain of 6.2 cm (or 0.95 SDS) in rhGH-treated patients with TS. Additionally, an adult height SDS ≥ −2 was reached by a higher rate of rhGH-treated patients in comparison with the untreated group (43 vs. 16%, $p < 0.001$). Adult height SDS within TH range (<1.6 SDS below TH SDS [13]) was also significantly more frequent in the rhGH-treated group than in the untreated group (52 vs. 15%, $p < 0.001$). Finally, adult height SDS corrected for TH SDS was higher in rhGH-treated (median −1.4, IQR −2.1, −0.9) than in untreated (median −2.5, IQR −3.0, −2.0, $p < 0.001$, Fig. 2b) patients, a difference that corresponds to 7.2 cm between these groups.

Table 1. Clinical data in rhGH-treated and untreated patients with TS

	Untreated group (n = 131)	rhGH-treated group			p value	
		all (n = 168)	adult height SDS <−2 (n = 96)	adult height SDS ≥−2 (n = 72)	untreated versus treated	AH SDS <−2 versus ≥−2
Year of birth	1983 (1977, 1987)	1993 (1987, 1997)	1994 (1988, 1997)	1992 (1987, 1995)	<0.001	0.230
TH SDS	−0.5 (−1.0, −0.1)	−0.9 (−1.5, −0.2)	−1.1 (−1.7, −0.5)	−0.5 (−1.1, −0.0)	<0.001	<0.001
Use of oxandrolone, %	0.8	7.1	9.4	4.2	0.016	0.320
Karyotype, %						
45,X	43.5	55.9	58.3	52.8	0.044	0.575
45,X/46,XX	21.4	6.5	4.2	9.7	<0.001	0.213
Y chromosome or Y-chromosome markers	11.4	6.5	5.2	8.3	0.198	0.620
Others	23.7	30.9	32.3	29.2	0.205	0.791
At first evaluation						
Chronological age, years	20.1 (18.0, 24.0)	11 (7.6, 13.1)	11.3 (7.7, 13.3)	10.6 (7.6, 12.8)	<0.001	0.277
Bone age, years	na	10 (6.8, 11)	10.0 (6.8, 11.0)	8.8 (6.9, 11.0)	na	0.641
Height SDS	−3.2 (−3.7, −2.4)	−3.2 (−3.8, −2.5)	−3.6 (−4.2, −2.9)	−2.7 (−3.2, −2.0)	0.670	<0.001
Height SDS – TH SDS	−2.5 (−3.0, −2.0)	−2.3 (−3.0, −1.7)	−2.5 (−3.3, −1.7)	−1.9 (−2.8, −1.7)	0.264	0.020
Predicted adult height SDS	na	−3.0 (−3.6, −2.4)	−3.4 (−4.0, −2.8)	−2.5 (−3.0, −1.9)	na	<0.001
At start of puberty						
Spontaneous puberty, %	17.6	23.4	26.1	20	0.327	0.474
Age at onset of spontaneous puberty, years	14.0 (12.7, 15)	12.4 (11.9, 13.9)	12.4 (11.9, 14.0)	12.7 (10.8, 13.9)	0.007	0.820
Age at onset of induced puberty, years	15 (14, 16)	14.4 (13, 2, 15.2)	14.4 (13.3, 15.2)	14.3 (13.2, 15.2)	0.003	0.831
At attainment of adult height						
rhGH duration, years	na	4.9 (3.5, 7.8)	4.7 (3.9, 7.0)	5.5 (3.9, 8.6)	na	0.068
rhGH dose, μg/kg/day	na	50 (46.3, 50)	50.0 (45.8, 50.0)	50.0 (47.5, 50.0)	na	0.833
Height, cm	142.8 (139, 148)	149 (144.5, 152.5)	145.5 (141.5, 147.7)	153.4 (151.4, 156.0)	<0.001	<0.001
Height SDS	−3.1 (−3.7, −2.3)	−2.2 (−2.9, −1.7)	−2.7 (−3.3, −2.4)	−1.5 (−1.8, −1.1)	<0.001	<0.001
Height SDS gain from first evaluation	0.01 (0.00, 0.01)	1.1 (0.3, 1.7)	0.8 (0.2, 1.5)	1.2 (0.6, 1.8)	<0.001	0.003
Height SDS gain from predicted adult height	na	0.8 (0.2, 1.4)	0.7 (0.1, 1.1)	1.1 (0.5, 1.4)	na	0.020
Height SDS – TH SDS	−2.5 (−3, −2)	−1.4 (−2.1, −0.9)	−1.9 (−2.4, −1.2)	−1.0 (−1.4, −0.3)	<0.001	<0.001
Height SDS ≥−2, %	16	42.9	0	100	<0.001	na

Data are shown as median and interquartile range or frequencies. The karyotype "others" includes isochromosome Xq and ring chromosome X. Predicted adult height was calculated according to the Bayley-Pinneau method [10]. TS, Turner syndrome; na, not available or not applicable; rhGH, recombinant human growth hormone; SDS, standard deviation score; TH, target height; AH, adult height.

Clinical Data in rhGH-Treated Patients according to Adult Height SDS

The comparison of clinical data between patients in the rhGH-treated group who reached an adult height SDS ≥ −2 and those who did not is also shown in Table 1. Patients who attained an adult height SDS ≥ −2 had a higher TH, were taller at the start of rhGH therapy, and had a higher first-year growth velocity SDS than patients who did not reach an adult height SDS ≥ −2. Karyotype, chronological and bone age in the beginning of rhGH treatment, treatment duration, rhGH dose, and pubertal data were not statistically different between the groups.

Combined height SDS at start of rhGH therapy and TH SDS positively predicted adult height SDS after treatment in a multiple linear regression model ($p < 0.001$, $R^2 = 0.375$). On the other hand, rhGH dose, treatment duration, age at start of rhGH therapy, age at start of puberty, presence of spontaneous puberty, and use of oxandrolone did not influence adult height in the present model.

Literature Review

After excluding duplicated studies, studies without a concomitant untreated group, and studies (or groups within studies) in which patients received rhGH for <2 years, or received oxandrolone, or received estrogens before 11 years old, we found 16 articles that compared adult height between groups of patients with TS who received rhGH treatment and who did not (Table 2).

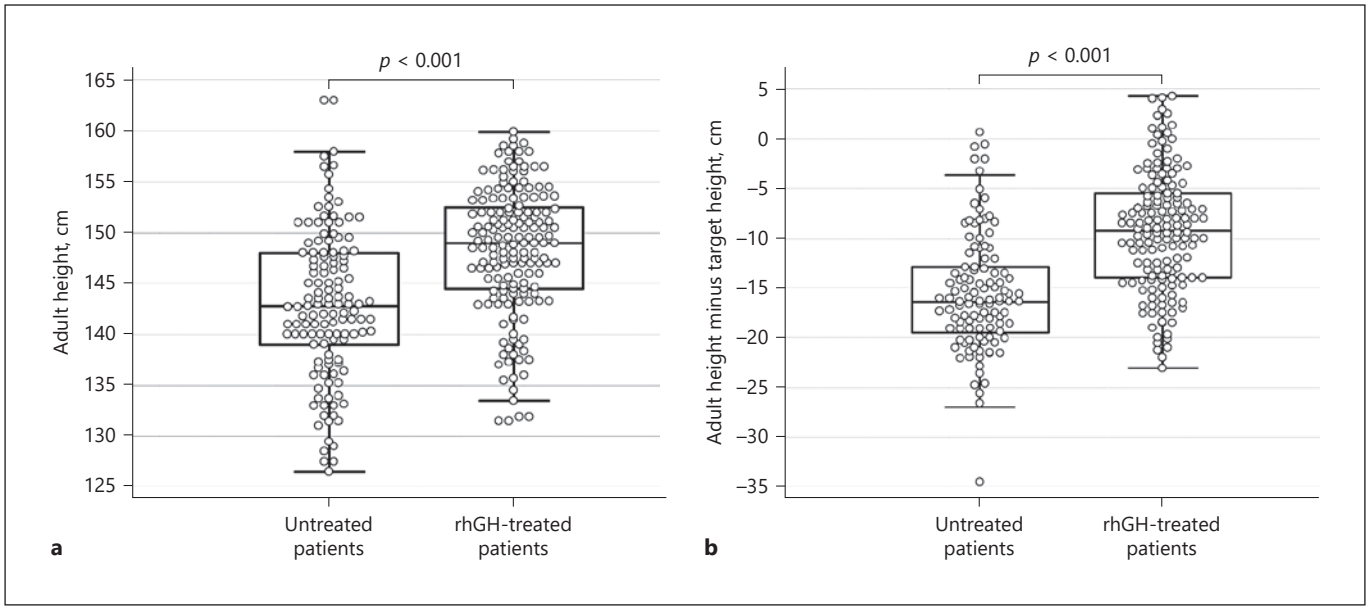

Fig. 2. Comparison of adult height (**a**) and adult height minus TH (**b**) between rhGH-treated and untreated patients with TS. TH, target height; TS, Turner syndrome.

In total, these studies evaluated 528 patients with TS treated with rhGH and 502 untreated patients. In rhGH-treated groups, the mean chronological age at the start of therapy was 10.8 years old, the mean rhGH dose was 0.3 mg/kg/week, and the mean duration of treatment was 5.0 years. The mean adult height SDS in patients with TS treated with rhGH was −2.2, and the mean difference in adult height between rhGH-treated and untreated patients was 5.9 cm. The factors that were most frequently correlated with adult height were TH and height at the start of rhGH (in SDS or cm).

When we analyzed our results together with these previous studies, a total of 696 rhGH-treated patients with TS were compared with 633 untreated patients. The mean results of the whole group were similar to the means of previous studies, with a mean difference in adult height between rhGH-treated and untreated patients of 5.7 cm (Table 2).

Discussion

Treatment of short stature with rhGH is considered to be safe and effective in TS [7]. Although there are only 2 randomized controlled studies evaluating adult height in patients with TS with and without rhGH treatment, many clinical trials are available, suggesting a mean height gain of 5–8 cm after rhGH (Table 2) [3]. Current recommendations are to start rhGH early (ideally between 4 and 6 years old) when there is evidence of growth failure, to use a rhGH dose of 45–50 μg/kg/day, and to maintain therapy until the patient is satisfied with her height or until there is little growth potential left (growth velocity <2 cm/year and bone age ≥14 years) [3].

However, there is considerable variability among the results of the available studies. This variability may arise from differences in patients' characteristics, such as age at start of rhGH therapy or midparental height, or from different treatment protocols, including rhGH dose or duration. Moreover, most studies were retrospective and had a relatively small cohort, and many of them did not have a concomitant untreated control group, basing their results on baseline-predicted adult height or historical controls. Our study, although also retrospective, evaluated a larger group of patients, treated with similar protocols, and had the opportunity to assess a concurrent untreated group. Additionally, we selected and reviewed previous studies that also reported rhGH effect in adult height in TS by comparing rhGH-treated patients with a parallel untreated sample. As a group, these studies support the effectiveness of rhGH therapy in TS patients, with a mean height gain over untreated patients and a mean adult height SDS confirming previous results in TS. Our larger and homogeneous sample brought results that were sim-

Table 2. Controlled studies about adult height after rhGH therapy in patients with TS [18–23, 25–34]

Study	Treated versus untreated, *n*	Adult height treated – untreated, cm	rhGH treatment				
			CA at the start, years	dose, mg/kg/week	duration, years	adult height SDS	predictors of adult height
Pasquino et al. [18]	18:18	5.4	13±2.0	0.17 → 0.33[a]	4.5±0.9	−2.4±1.2[b]	TH
Taback et al. [27]	17:14	7.3	12.4 (10.1, 13.8)	0.3	3.6	−2.3[c]	na
Dacou-Voutetakis et al. [19]	35:27	2.1	12.0±1.8	0.23±0.07	2.7±1.2	−2.6±1.0[b]	Height SDS and BA at rhGH start, TH, birth weight
Rosenfeld et al. [28]	17:25	7.4	9.1±2.1	0.375	7.6±2.2	−2.0±0.9[c]	na
Hochberg and Zadik [20]	25:24	4.4	10.7±1.4	0.273	5.1±1.9	−2.4±0.8[c]	TH
Stephure et al. [29]	61:43[d]	7.2	10.3±1.8	0.3	5.7±1.6	−2.4±1.0[b]	CA at rhGH start
Pasquino et al. [21]	60:59	6.8	10.9±2.8	0.33	6.8	−1.9±1.0[b]	TH, height at rhGH start
Bechtold et al. [22]	44:12	2.8	10.9±3.0	0.33	5.2±2.5	−2.0±0.9[b]	CA at rhGH start, duration of rhGH therapy, height SDS and CA at pubertal onset, TH
Hsu et al. [23]	21:28	5.4	11.5±1.8	0.33	4.0±1.5	−1.7±0.9[e]	TH, height, and GV at rhGH start, first-year GV
Morin et al. [25]	25:10	9.6	13.0 (5.6, 15.8)	0.33±0.06	3.8 (2.1, 10.3)	−1.8±1.1[e]	Height SDS and CA at rhGH start, duration of rhGH treatment before estrogen onset
Baldin et al. [30]	30:52	2.3	10.0±1.3	0.42 (0.32, 0.5)	3.7±1.5	−2.6±0.6[c]	na
Ross et al. [31]	27:17[d]	3.3	8.4±2.7	0.3	7.4±2.8	−2.3±1.1	na
Hoxha et al. [32]	25:27	2.9	12.1±3.6	0.28	3.3	−2.9±0.8[c]	na
Sánchez Marco et al. [26]	17:8	10.7	7.9±4.1	0.34±0	7.4±3.9	−1.2±0.6	rhGH dose, duration of rhGH treatment before estrogen onset, first-year increment in IGF-1 and IGFBP3 and first-year GV
Irzyniec et al. [33]	33:124	4.9	12±3.5	0.33–0.47	3.5±2.4	−2.3±1.0[c]	na
Ahn et al. [34]	73:14	8.5	8.9±3.7	0.33	6.5±3.0	−1.9±1.0[e]	Height SDS at rhGH start
Present study	168:131	6.2	10.5±3.6	0.33±0.04	5.8±2.8	−2.3±1.0	TH, height SDS at rhGH start
All studies	696:633[f]	5.7[g]	10.8[g]	0.34[g]	5.1[g]	−2.2[g]	TH and height SDS at rhGH start[h]

Data are shown as mean ± standard deviation or median (range). TS, Turner syndrome; BA, bone age; CA, chronological age; GV, growth velocity; na, not available or not applicable; rhGH, recombinant human growth hormone; SDS, standard deviation score; TH, target height. [a] rhGH dose was 0.17 mg/kg/week during the first year of treatment and 0.33 mg/kg/week thereafter. [b] Adult height SDS calculated according to growth charts for girls with TS was recalculated according to the Centers for Disease Control and Prevention (CDC) growth charts. [c] Adult heights described in centimeters were converted to adult height SDS according to CDC growth charts. [d] Randomized controlled studies. [e] Adult height SDS calculated according to local growth charts was not converted to adult height SDS according to CDC growth charts. [f] Total number of rhGH-treated and untreated patients evaluated in the studies. [g] Mean values in the studies. [h] Factors that were most frequently correlated with adult height.

ilar to those means, reinforcing the findings in this group and adding accuracy to these data.

Besides the variability of results among studies, individual responses to rhGH therapy are also highly variable, and, consequently, many researchers tried to find predictive factors of adult height after rhGH treatment. In our group, TH SDS and height SDS at the onset of rhGH treatment were predictors of adult height SDS, and these 2 characteristics were the main predictive factors of adult height according to our literature review. In that, other

predictors found in more than one study were chronological age at rhGH start and duration of rhGH therapy before pubertal onset, both emphasizing the importance of an early diagnosis of TS to allow an early start of rhGH therapy and an age-appropriate induction of puberty.

One limitation of our study is the median difference of a decade in the year of birth between rhGH-treated and untreated patients with TS, which may have influenced our results due to the secular height trends. In 2018, Woelfle et al. [14] evaluated secular trends in patients with TS in a retrospective analysis of KIGS data. Among other factors, they compared height SDS of prepubertal, untreated, 8-year-old patients with TS born in different years and found a 0.27 higher height SDS in those born in the period 1990–1994 compared to those born in 1980–1984, which is equivalent to approximately 1.5 cm [14]. A Brazilian study on secular height trends in healthy adolescents showed a mean increment of height of approximately 0.2 cm/year from 1974 to 2003 or 2 cm in a decade [15]. However, the 6.2-cm adult height gain seen in our rhGH-treated group surpassed secular trends. Moreover, adult height SDS corrected for TH SDS, which accounts for part of the secular height trend, was also significantly higher in the rhGH-treated group, estimating the height gain in 7.2 cm.

Another factor that could have improved the height gain response in the rhGH-treated group was the use of oxandrolone in 7.1% ($n = 12$) patients. Oxandrolone is an anabolic steroid and was previously associated with height gain in patients with TS [16]. Li et al. [17] reported in their meta-analysis that its association with rhGH treatment increased final height in patients with TS by 2.46 cm. However, its use is still controversial because of its potential risks of delayed breast development and dose-dependent virilization [3]. In our linear regression analysis, there was no correlation between the use of oxandrolone and adult height, maybe because of the small number of patients who received it in the present study.

On the other hand, TH, which was considered a predictor of adult height in our group and in many previous studies [18–23], was significantly lower in our rhGH-treated group. Moreover, there was a significant difference in the distribution of karyotypes between the groups, and the rhGH-treated group had significantly more patients with 45,X karyotype and significantly less patients with 45,X/46,XX karyotype. Although individual findings are highly variable, as a group, patients with TS and 45,X karyotype tend to be shorter than patients with 45,X/46,XX karyotype [24]. Finally, the rhGH-treated group was significantly younger at onset of spontaneous or induced puberty, which is a factor negatively associated with adult height SDS in previous studies [22, 25, 26], despite not reaching significance in our patients. In conclusion, the results from our cohort together with the results from the studies in our literature review strengthen the evidence for the efficacy of rhGH therapy in patients with TS from different populations.

Statement of Ethics

The study protocol was approved by the Research Ethics Committees of HC-FMUSP, HC-FCM-UNICAMP, and HC-FMRP-USP (Study Approval Reference No. 1.684.978). Written informed consent was obtained from all patients.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

N.C.B.D. made substantial contributions to acquisition and analysis of data and helped drafting the manuscript. A.F.B., A.M., S.L.M., E.R.S., S.R.A., and G.G.J. made substantial contributions to acquisition of data and revised it critically for important intellectual content. I.J.P.A. and B.M. made substantial contributions to the conception of the work and revised it critically for important intellectual content. A.J. and R.C.S. made substantial contributions to the conception of the work, acquisition, analysis, and interpretation of data, and helped drafting the manuscript. All authors approved the final submitted version and agreed to be accountable for the accuracy and integrity of the work.

References

1 Ford C, Jones K, Polani P, De Almeida J, Briggs J. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet. 1959 Apr;1(7075):711–3.
2 Nielsen J, Wohlert M. Chromosome abnormalities found among 34910 newborn children: results from a 13-year incidence study in Århus, Denmark. Hum Genet. 1991;87(1):81–3.

3 Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, et al. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. Eur J Endocrinol. 2017 Sep;177(3):G1–G70.
4 Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, et al. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat Genet. 1997 May;16(1):54–63.
5 Ranke MB, Pflüger H, Rosendahl W, Stubbe P, Enders H, Bierich JR, et al. Turner syndrome: spontaneous growth in 150 cases and review of the literature. Eur J Pediatr. 1983 Dec;141(2):81–8.
6 Lyon AJ, Preece MA, Grant DB. Growth curve for girls with Turner syndrome. Arch Dis Child. 1985 Oct;60(10):932–5.
7 Los E, Rosenfeld RG. Growth and growth hormone in Turner syndrome: looking back, looking ahead. Am J Med Genet C Semin Med Genet. 2019 Mar;181(1):86–90.
8 Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969 Jun;44(235):291–303.
9 Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, et al. 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat 11. 2002 May;(246):1–190.
10 Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. Am J Med Sci. 1959 Sep;238(3):393.
11 Bayley N, Pinneau SR. Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standards. J Pediatr. 1952 Apr;40(4):423–41.
12 Tanner JM, Goldstein H, Whitehouse RH. Standards for children's height at age 2 to 9 years allowing for height of parents. Arch Dis Child. 1970 Dec;45(244):819–62.
13 Wit JM, Kamp GA, Oostdijk W; on behalf of the Dutch Working Group on Triage and Diagnosis of Growth Disorders in Children. Towards a rational and efficient diagnostic approach in children referred for growth failure to the general paediatrician. Horm Res Paediatr. 2019;91(4):223–40.
14 Woelfle J, Lindberg A, Aydin F, Ong KK, Camacho-Hubner C, Gohlke B. Secular trends on birth parameters, growth, and pubertal timing in girls with Turner syndrome. Front Endocrinol. 2018 Feb;9:54.
15 Conde WL, Rinaldi AEM, Enes CC. Is the secular trend in height delaying overweight rise among adolescents? The Brazilian case. Public Health Nutr. 2016 Aug;19(12):2213–9.
16 Menke LA, Sas TC, de Muinck Keizer-Schrama SM, Zandwijken GR, de Ridder MA, Odink RJ, et al. Efficacy and safety of oxandrolone in growth hormone-treated girls with Turner syndrome. J Clin Endocrinol Metab. 2010 Mar;95(3):1151–60.
17 Li P, Cheng F, Xiu L. Height outcome of the recombinant human growth hormone treatment in Turner syndrome: a meta-analysis. Endocr Connect. 2018 Apr;7(4):573–83.
18 Pasquino AM, Passeri F, Municchi G, Segni M, Pucarelli I, Larizza D, et al. Final height in Turner syndrome patients treated with growth hormone. Horm Res. 1996;46(6):269–72.
19 Dacou-Voutetakis C, Karavanaki-Karanassiou K, Petrou V, Georgopoulos N, Maniati-Christidi M, Mavrou A. The growth pattern and final height of girls with Turner syndrome with and without human growth hormone treatment. Pediatrics. 1998 Apr;101(4 Pt 1):663–8.
20 Hochberg Z, Zadik Z. Final height in young women with Turner syndrome after GH therapy: an open controlled study. Eur J Endocrinol. 1999 Sep;141(3):218–24.
21 Pasquino AM, Pucarelli I, Segni M, Tarani L, Calcaterra V, Larizza D. Adult height in sixty girls with Turner syndrome treated with growth hormone matched with an untreated group. J Endocrinol Invest. 2005 Apr;28(4):350–6.
22 Bechtold S, Dalla Pozza R, Schmidt H, Bonfig W, Schwarz HP. Pubertal height gain in Ullrich-Turner syndrome. J Pediatr Endocrinol Metab. 2006 Aug;19(8):987–93.
23 Hsu P-Y, Tung Y-C, Tsai W-Y, Lee J-S, Hsiao P-H. Effect of growth hormone therapy on adult height of children with Turner syndrome. J Formos Med Assoc. 2008 Sep;107(9):704–9.
24 Fiot E, Zenaty D, Boizeau P, Haigneré J, Dos Santos S, Léger J, et al. X-chromosome gene dosage as a determinant of impaired pre and postnatal growth and adult height in Turner syndrome. Eur J Endocrinol. 2016 Mar;174(3):281–8.
25 Morin A, Guimarey LM, Apezteguia M, Santucci ZC. Adult height in Turner Syndrome girls after long-term growth hormone treatment. Medicina (B Aires). 2009;69(4):431–6.
26 Sánchez Marco SB, de Arriba Muñoz A, Ferrer Lozano M, Labarta Aizpún JI, Garagorri Otero JM. [Human growth hormone and Turner syndrome]. An Pediatr. 2017 Feb;86(2):81–6.
27 Taback SP, Collu R, Deal CL, Guyda HJ, Salisbury S, Dean HJ, et al. Does growth-hormone supplementation affect adult height in Turner's syndrome? Lancet. 1996 Jul;348(9019):25–7.
28 Rosenfeld RG, Attie KM, Frane J, Brasel A, Burstein S, Cara JF, et al. Growth hormone therapy of Turner's syndrome: beneficial effect on adult height. J Pediatr. 1998 Feb;132(2):319–24.
29 Stephure DK; Canadian Growth Hormone Advisory Committee. Impact of growth hormone supplementation on adult height in Turner syndrome: results of the Canadian randomized controlled trial. J Clin Endocrinol Metab. 2005 Jun;90(6):3360–6.
30 Baldin AD, Fabbri T, Siviero-Miachon AA, Spinola-Castro AM, de Lemos-Marini SH, Baptista MT, et al. Growth hormone effect on body composition in Turner syndrome. Endocrine. 2011 Dec;40(3):486–91.
31 Ross JL, Quigley CA, Cao D, Feuillan P, Kowal K, Chipman JJ, et al. Growth hormone plus childhood low-dose estrogen in Turner's syndrome. N Engl J Med. 2011 Mar;364(13):1230–42.
32 Hoxha P, Babameto-Laku A, Vyshka G, Gjoka K, Minxuri D, Myrtaj E, et al. Turner syndrome in Albania and the efficacy of its treatment with growth hormone. J Pediatr Endocrinol Metab. 2015 Jan;28(11–12):1227.
33 Irzyniec T, Jeż W, Lepska K, Maciejewska-Paszek I, Frelich J. Childhood growth hormone treatment in women with Turner syndrome: benefits and adverse effects. Sci Rep. 2019 Dec;9(1):15951.
34 Ahn JM, Suh JH, Kwon AR, Chae HW, Kim HS. Final adult height after growth hormone treatment in patients with Turner syndrome. Horm Res Paediatr. 2019;91(6):373–9.

4 DISCUSSÃO

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4.1 FATORES GENÉTICOS IMPLICADOS NA VARIABILIDADE FENOTÍPICA NOS INDIVÍDUOS COM HAPLOINSUFICIÊNCIA DO *SHOX*

O fenótipo associado a haploinsuficiência do *SHOX* é caracterizado pela variabilidade fenotípica que ocorre mesmo entre indivíduos da mesma família com a mesma alteração gênica[31]. Em indivíduos com alterações restritas a região regulatória do *SHOX* essa variabilidade fenotípica se torna ainda mais expressiva devido à baixa penetrância associada a esses defeitos[34]. No nosso estudo, avaliamos 98 indivíduos que representavam extremos do fenótipo dentro das famílias com alteração do *SHOX* e indivíduos com alterações restritas as regiões regulatórias.

Nós encontramos uma segunda variante patogênica segregando com o fenótipo mais grave em 4 das 48 famílias analisadas. Essas variantes foram encontradas nos genes citocromo P450 família 26 subfamília C membro 1 (*CYP26C1,* MIM *608428*), aggrecan (ACAN,* MIM *155760) e hormônio semelhante ao hormônio paratireóide *(PTHLH,* MIM *168470), genes da placa de crescimento que são diretamente ou indiretamente relacionados a mesma via que o gene *SHOX*. Também foram identificadas variantes de interesse nos genes receptor do peptídeo natriurético B *(NPR2,* MIM * 607072*), runt related transcription factor 2 (RUNX2,* MIM * 600211*) e (TP53,* MIM * 191170*).* Acreditamos que alterações nesses genes possam intensificar a desregulação dos condrócitos nas zonas da placa de crescimento relacionada a alteração primária do gene *SHOX* levando a um fenótipo mais grave.

Até o momento, somente um estudo havia analisado a presença de outras variantes gênicas modulando o fenótipo na haploinsuficiência do *SHOX*. Nesse estudo, Montalbano e colaboradores[58], estudando a variabilidade fenotípica em pacientes com alteração do *SHOX*, analisou uma grande família afetada com DLW por exoma e identificou uma variante no gene *CYP26C1* que segregava com o

fenótipo mais grave. Adicionalmente, este estudo analisou o gene *CYP26C1* por sanger em 68 indivíduos com DLW e identificou duas outras variantes *missense* neste gene em mais duas famílias com alteração do *SHOX*, também segregando com o fenótipo mais grave. O gene *CYP26C1* codifica um membro da família do citocromo P450 envolvido no catabolismo do ácido retinóico (AR). O ácido retinóico atua como fator inibidor da expressão do *SHOX*[58]. Através de estudos funcionais, este estudo demostrou que as variantes encontradas no *CYP26C1* levam a uma perda de função desse gene que, indiretamente através do excesso do AR, diminui a expressão do *SHOX*. Comprovando que alterações no *CYP26C1* tem papel modular do fenótipo nos defeitos do *SHOX*, causando um fenótipo mais grave. Na nossa casuística, alterações relacionadas a esse gene foram implicadas como moduladoras do fenótipo em 2 das 48 famílias (4%) analisadas, prevalência semelhante a encontrada por Montalbano e colaboradores[58] na sua coorte.

Nas famílias com alterações isoladas da região regulatória, uma segunda variante patogênica foi encontrada em 27% (em 3 de 11 famílias) das famílias analisadas, prevalência maior do que nas famílias com alteração envolvendo a região codificadora do gene. Indivíduos com alterações isoladas da região regulatória do *SHOX*, em geral, tem um fenótipo mais leve, apresentando menor grau de desproporção corporal e/ou deformidade de Madelung, quando comparadas a alterações envolvendo diretamente o gene *SHOX*[20,34]. Levantando o questionamento sobre o real papel patogênico dessas variantes isoladamente em causar o fenótipo. Nossa proposição é que a presença de uma segunda variante seja importante para determinar a penetrância e expressão do fenótipo em indivíduos com alterações isoladas da região regulatória.

Na maioria dos centros médicos, a investigação de baixa estatura se inicia pela investigação genética de alterações no gene *SHOX* e/ou sua região regulatória por amplificação dependente da ligação de múltiplas sondas (MLPA) e/ou sequenciamento por sanger pela alta prevalência de alterações relacionadas a esse gene nessa população[42]. Diante da presença de um achado positivo, os pacientes não são submetidos a testes genéticos adicionais. Dessa forma, não é possível identificar a presença de outras variantes modulando o fenótipo. Nos últimos anos, o acesso ao

sequenciamento de nova geração aumentou substancialmente, facilitando a análise desses pacientes por tecnologias com análise mais ampla. Acreditamos que com uma avaliação genética mais ampla desses indivíduos, será possível entender melhor os fatores que influenciam na variabilidade fenotípica e penetrância dentro dessas famílias com haploinsuficiência do *SHOX*.

Atualmente, o conhecimento sobre modificadores genéticos tem se expandindo em diversas áreas da medicina[44]. Na literatura de baixa estatura, fatores genéticos que podem modular o fenótipo ainda são motivos de estudo. Nosso estudo trouxe importante contribuição para a literatura de baixa estatura, pois expande o conhecimento sobre a variabilidade fenotípica em indivíduos com alterações do *SHOX* e levanta o questionamento sobre a interação de variantes patogênicas na mesma via na determinação do fenótipo desse gene.

4.2 ALTURA ADULTA NOS INDIVÍDUOS COM ALTERAÇÃO DO *SHOX*

4.2.1 Haploinsuficiência do *SHOX*

O tratamento da baixa estatura associada a haploinsuficiência do *SHOX* com GH já é bem estabelecido, sendo baseado principalmente na resposta ao tratamento com GH na Síndrome de Turner e em estudos clínicos em indivíduos com haploinsuficiência do *SHOX*[53,54,60]. Porém, a maioria dos estudos não descreve a altura adulta atingida após tratamento com GH e nem compara a altura adulta de indivíduos tratados com um grupo controle não tratado, baseando seus resultados na previsão de altura adulta calculada no início do tratamento[50,53,54].

Neste estudo, analisamos uma coorte de 39 indivíduos com haploinsuficiência do *SHOX* que atingiram altura adulta. Nós comparamos a altura adulta de indivíduos tratados com um grupo controle não tratado com GH. Nosso estudo confirmou a efetividade do tratamento com GH em melhorar a altura adulta dos indivíduos com

SHOX usando evidências de vida real. Os indivíduos tratados tiveram altura adulta mediana de 1,0 desvio-padrão maior que os indivíduos não tratados, ou seja, 6,3 cm mais altos. Enquanto a prevalência da baixa estatura no grupo tratado com GH diminui ao longo do acompanhamento, no grupo não tratado, essa prevalência passou de 31% no início para 77% no final do seguimento.

Outra contribuição importante do nosso artigo para a literatura, é a descrição do crescimento desses pacientes durante a puberdade. No grupo controle foi observado queda do desvio-padrão da altura durante este período. Pacientes que no início da puberdade tinham altura no limite inferior da normalidade perdem desvio-padrão da altura nessa fase, atingindo uma altura adulta menor que -2,0 desvios-padrões. Ressaltamos a importância do acompanhamento cuidadoso dessas crianças com altura no limite inferior da normalidade durante toda a fase de crescimento, mas principalmente na puberdade, para que não se perca a janela de oportunidade de início do tratamento com GH para baixa estatura.

No nosso estudo, o grupo de pacientes que começou o tratamento no início da puberdade e/ou tinham pior predição de altura adulta no início do tratamento usaram GH associado a análogos de hormônio liberador de gonadotrofina (aGnRH). Esses pacientes conseguiram atingir altura adulta semelhante ao grupo de pacientes que iniciou o tratamento pré-púbere e fez uso isolado de GH. Esses pacientes tiveram um ganho de altura adulta em relação à altura prevista no início do tratamento maior do que o grupo que fez tratamento isolado com GH. Indicando que o tratamento combinado com GH e aGnRH é uma boa opção para melhorar a altura adulta nos indivíduos que iniciam o tratamento tardiamente, no início da puberdade, ou que tem predição de altura final ruim no início do tratamento.

4.2.2 Síndrome de Turner

Nesse estudo tivemos a oportunidade de analisar uma grande coorte brasileira de pacientes com ST acompanhadas em 3 centros diferentes de serviços terciários

em Endocrinologia e Metabologia. Nosso estudo mostrou que pacientes tratadas com GH atingiram melhor altura adulta que as não tratadas com uma diferença mediana de altura de 6 cm entre os grupos. Comparando nossos resultados com estudos prévios com metodologia semelhante, apesar das diferenças entre as casuísticas das características clínicas e das respostas com o tratamento com GH, como grupo os estudos mostraram o benefício com a medicação no ganho de altura adulta na ST. O artigo trouxe importante contribuição para a literatura confirmando a eficácia do tratamento com GH na ST através de evidências de mundo real.

Nosso estudo também mostrou que a altura no início do tratamento com GH e a altura alvo são parâmetros que foram preditores da altura final, assim como a idade de início do tratamento e o tempo de tratamento com GH, evidenciados por outros estudos[61,62]. Usando esses dados, podemos alinhar as expectativas em relação ao tratamento com GH das famílias com a resposta esperada em relação à altura adulta.

4.2.3 Hormônio do crescimento na haploinsuficiência do *SHOX* e na Síndrome de Turner: comparativo

A baixa estatura é mais grave em pacientes com ST do que em pacientes com haploinsuficiência do *SHOX*, sugerindo mecanismos adicionais além da alteração do *SHOX* que comprometem o crescimento em pacientes com ST[10]. Quando comparamos a resposta ao tratamento com GH nesses dois grupos de pacientes, os pacientes com ST tiveram maior ganho de DP da altura adulta em relação à altura inicial após tratamento com GH quando comparados com os pacientes com haploinsuficiência do *SHOX* (ganho mediana de DP de 1,1 e 0,6, respectivamente). Porém, sua altura adulta ainda persistiu pior que a dos pacientes com haploinsuficiência do *SHOX* (DP da altura adulta -2,2 e -1,6, respectivamente).

Comparando os resultados dos capítulos 2 e 3, os pacientes com ST tem baixa estatura mais grave, porém melhor resposta ao tratamento com GH quando comparados com pacientes com haploinsuficiência do *SHOX*. Esse resultado foi

diferente do observado em estudos prévios, onde o ganho de DP da altura adulta em relação à altura inicial foi semelhante entre os grupos com ST e haploinsuficiência do *SHOX*[54,60]. Isso pode ser explicado devido parte da nossa casuística com haploinsuficiência do *SHOX* ter iniciado o tratamento com GH próximo ao período de início da puberdade. Adicionalmente, o hipogonadismo associado a ST e o consequente retardo em iniciar a indução puberal podem ser outros fatores que expliquem o maior ganho de altura neste grupo.

5 CONCLUSÕES

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- Variantes patogênicas em genes relacionados a placa de crescimento atuam como moduladores do fenótipo na haploinsuficiência do gene *SHOX*. Nos indivíduos com alterações restritas as regiões regulatórias, a presença de uma segunda variante patogênica pode ter papel fundamental na determinação da penetrância e expressão do fenótipo.

- O tratamento com hormônio do crescimento é efetivo em melhorar a altura adulta dos pacientes com haploinsuficiência do *SHOX*. A terapia combinada com GH e aGnRH melhora os resultados em relação à altura adulta em crianças com diagnóstico tardio ou predição de altura adulta ruim. Crianças com haploinsuficiência do *SHOX* e altura no limite inferior da normalidade necessitam de monitoramento do crescimento devido ao risco de piora da altura durante a puberdade.

- A terapia com hormônio do crescimento foi eficaz em promover ganho de altura nos pacientes com Síndrome de Turner.

REFERÊNCIAS

1. Marchini A, Ogata T, Rappold GA. A track record on SHOX: from basic research to complex models and therapy. Endocr Rev. 2016;37(4):417-48. doi: 10.1210/er.2016-1036.

2. Toews MW. File: Standard deviation diagram.svg [Internet]. [place unknown]: Wikimedia Commons; 2023 [cited 2023 Nov 24]. Available from: https://commons.wikimedia.org/w/index.php?title=File:Standard_deviation_diagram.svg&oldid=769672706

3. Visscher PM. Commentary: Height and Mendel's theory: the long and the short of it. Int J Epidemiol. 2013;42(4):944-5. doi: 10.1093/ije/dyt069.

4. Wit JM, et al. Mechanisms in endocrinology: novel genetic causes of short stature. Eur J Endocrinol. 2016;174(4):R145-73. doi: 10.1530/EJE-15-0937.

5. Durand C, Rappold GA. Height matters-from monogenic disorders to normal variation. Nat Rev Endocrinol. 2013;9(3):171-7. doi: 10.1038/nrendo.2012.251.

6. Baron J, et al. Short and tall stature: a new paradigm emerges. Nat Rev Endocrinol. 2015;11(12):735-46. doi: 10.1038/nrendo.2015.165.

7. Jacobs PA, et al. Cytogenetic studies in primary amenorrhea. Lancet. 1961;1(7188):1183-9. doi: 10.1016/S0140-6736(61)91939-0.

8. Fisher EM, et al. Homologous ribosomal protein genes on the human X and Y chromosomes: escape from X inactivation and possible implications for Turner syndrome. Cell. 1990;63(6):1205-18. doi: 10.1016/0092-8674(90)90416-c.

9. Gianfrancesco F, et al. A novel pseudoautosomal gene encoding a putative GTP-binding protein resides in the vicinity of the Xp/Yp telomere. Hum Mol Genet. 1998;7(3):407-14. doi: 10.1093/hmg/7.3.407.

10. Rao E, et al. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat Genet. 1997;16(1):54-63. doi: 10.1038/ng0597-54.

11. Ellison JW, et al. PHOG, a candidate gene for involvement in the short stature of Turner syndrome. Hum Mol Genet. 1997;6(8):1341-7. doi: 10.1093/hmg/6.8.1341.

12. Marchini A, et al. The short stature homeodomain protein SHOX induces cellular growth arrest and apoptosis and is expressed in human growth plate chondrocytes. J Biol Chem. 2004;279(35):37103-14. doi: 10.1074/jbc.M307006200.

13. Rao E, et al. The Leri-Weill and Turner syndrome homeobox gene SHOX encodes a cell-type specific transcriptional activator. Hum Mol Genet. 2001;10(26):3083-91. doi: 10.1093/hmg/10.26.3083.

14. Durand C, et al. Alternative splicing and nonsense-mediated RNA decay contribute to the regulation of SHOX expression. PLoS One. 2011;6(3):e18115. doi: 10.1371/journal.pone.0018115.

15. Munns CJF, et al. Expression of SHOX in human fetal and childhood growth plate. J Clin Endocrinol Metab. 2004;89(8):4130-5. doi: 10.1210/jc.2003-032230.

16. Blaschke RJ, et al. Transcriptional and translational regulation of the Leri-Weill and Turner syndrome homeobox gene SHOX. J Biol Chem. 2003;278(48):47820-6. doi: 10.1074/jbc.M306685200.

17. Benito-Sanz S, et al. A novel class of pseudoautosomal region 1 deletions downstream of SHOX is associated with Léri-Weill dyschondrosteosis. Am J Hum Genet. 2005;77(4):533-44. doi: 10.1086/449313.

18. Fukami M, et al. Transactivation function of an approximately 800-bp evolutionarily conserved sequence at the SHOX 3′ region: implication for the downstream enhancer. Am J Hum Genet. 2006;78(1):167-70. doi: 10.1086/499254.

19. Sabherwal N, et al. Long-range conserved non-coding SHOX sequences regulate expression in developing chicken limb and are associated with short stature phenotypes in human patients. Hum Mol Genet. 2007;16(2):210-22. doi: 10.1093/hmg/ddl470.

20. Benito-Sanz S, et al. Identification of the first recurrent PAR1 deletion in Léri-Weill dyschondrosteosis and idiopathic short stature reveals the presence of a novel SHOX enhancer. J Med Genet. 2012;49(7):442-50. doi: 10.1136/jmedgenet-2011-100678.

21. Durand C, et al. Enhancer elements upstream of the SHOX gene are active in the developing limb. Eur J Hum Genet. 2010;18(5):527-32. doi: 10.1038/ejhg.2009.216.

22. Kronenberg HM. Developmental regulation of the growth plate. Nature. 2003;423(6937):332-6. doi: 10.1038/nature01657.

23. Decker E, et al. FGFR3 is a target of the homeobox transcription factor SHOX in limb development. Hum Mol Genet. 2011;20(8):1524-35. doi: 10.1093/hmg/ddr030.

24. Munns CF, et al. Histopathological analysis of Leri-Weill dyschondrosteosis: disordered growth plate. Hand Surg. 2001;6(1):13-23. doi: 10.1142/s0218810401000424.

25. Filatov DA, Gerrard DT. High mutation rates in human and ape pseudoautosomal genes. Gene. 2003;317(1-2):67-77. doi: 10.1016/s0378-1119(03)00697-8.

26. Rouyer F, et al. A gradient of sex linkage in the pseudoautosomal region of the human sex chromosomes. Nature. 1986;319(6051):291-5. doi: 10.1038/319291a0.

27. Funari MFA, et al. Usefulness of MLPA in the detection of SHOX deletions. Eur J Med Genet. 2010;53(5):234-8. doi: 10.1016/j.ejmg.2010.06.001.

28. Fokkema IFAC, et al. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat. 2011;32(5):557-63. doi: 10.1002/humu.21438.

29. Binder G, Schwarze CP, Ranke MB. Identification of short stature caused by SHOX defects and therapeutic effect of recombinant human growth hormone. J Clin Endocrinol Metab. 2000;85(1):245-9. doi: 10.1210/jcem.85.1.6375.

30. Blum WF, et al. Growth Hormone is effective in treatment of short stature associated with short stature homeobox-containing gene deficiency: two-year results of a randomized, controlled, multicenter trial. J Clin Endocrinol Metab. 2007;92(1):219-28. doi: 10.1210/jc.2006-1409.

31. Jorge AAL, et al. SHOX mutations in idiopathic short stature and Leri-Weill dyschondrosteosis: frequency and phenotypic variability. Clin Endocrinol (Oxf). 2007;66(1):130-5. doi:10.1111/j.1365-2265.2006.02698.x.

32. Binder G, et al. SHOX Haploinsufficiency and Leri-Weill dyschondrosteosis: prevalence and growth failure in relation to mutation, sex, and degree of wrist deformity. J Clin Endocrinol Metab. 2004;89(9):4403-8. doi: 10.1210/jc.2004-0591.

33. Belin V, et al. SHOX mutations in dyschondrosteosis (Leri-Weill syndrome). Nat Genet. 1998;19(1):67-9. doi: 10.1038/ng0198-67.

34. Rosilio M, et al. Genotypes and phenotypes of children with SHOX deficiency in France. J Clin Endocrinol Metab. 2012;97(7):E1257-E1265. doi: 10.1210/jc.2011-3460.

35. Zinn AR, et al. Complete SHOX deficiency causes Langer mesomelic dysplasia. Am J Med Genet. 2002;110(2):158-63. doi: 10.1002/ajmg.10422.

36. Upners EN, et al. Short stature homeobox-containing gene duplications in 3.7% of girls with tall stature and normal karyotypes. Acta Paediatr. 2017;106(10):1651-7. doi: 10.1111/apa.13969.

37. Tüttelmann F, Gromoll J. Novel genetic aspects of Klinefelter's syndrome. Mol Hum Reprod. 2010;16(6):386-95. doi: 10.1093/molehr/gaq019.

38. Benito-Sanz S, et al. Clinical and molecular evaluation of SHOX/PAR1 duplications in Leri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS). J Clin Endocrinol Metab. 2011;96(2):E404-12. doi: 10.1210/jc.2010-1689.

39. Malaquias AC, 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):449-56. doi: 10.1159/000355411.

40. Langer Jr. LO. Dyschondrosteosis, a hereditable bone dysplasia with characteristic roentgenographic features. Am J Roentgenol Radium Ther Nucl Med. 1965;95(1):178-88. doi: 10.2214/ajr.95.1.178.

41. Ross JL, et al. Phenotypes associated with SHOX deficiency. J Clin Endocrinol Metab. 2001;86(12):5674-80. doi: 10.1210/jcem.86.12.8125.

42. Rappold G, et al. Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. J Med Genet. 2007;44(5):306-13. doi: 10.1136/jmg.2006.046581.

43. Joustra SD, et al. Novel clinical criteria allow detection of short stature Homeobox-containing gene haploinsufficiency caused by either gene or enhancer region defects. Horm Res Paediatr. 2019;92(6):372-81. doi: 10.1159/000507215.

44. Kousi M, Katsanis N. Genetic modifiers and oligogenic inheritance. Cold Spring Harb Perspect Med. 2015;5(6):a017145. doi: 10.1101/cshperspect.a017145.

45. Riordan JD, Nadeau JH. From peas to disease: modifier genes, network resilience, and the genetics of health. Am J Hum Genet. 2017;101(2):177-91. doi: 10.1016/j.ajhg.2017.06.004.

46. Nadeau JH. Modifier genes in mice and humans. Nat Rev Genet. 2001;2(3):165-74. doi: 10.1038/35056009.

47. Dantas NCB, et al. Identification of a second genetic alteration in patients with SHOX deficiency individuals: a potential explanation for phenotype variability. Eur J Endocrinol. 2023;189(3):387-95. doi: 10.1093/ejendo/lvad128.

48. Lyon AJ, Preece MA, Grant DB. Growth curve for girls with Turner syndrome. Arch Dis Child. 1985;60(10):932-5. doi: 10.1136/adc.60.10.932.

49. Los E, Rosenfeld RG. Growth and growth hormone in Turner syndrome: lo-oking back, looking ahead. Am J Med Genet C Semin Med Genet. 2019;181(1):86-90. doi: 10.1002/ajmg.c.31680.

50. Benabbad I, et al. Safety outcomes and near-adult height gain of growth hormone-treated children with SHOX deficiency: data from an observational study and a clinical trial. Horm Res Paediatr. 2017;87(1):42-50. doi: 10.1159/000452973.

51. Salmon-Musial AS, et al. Clinical and radiological characteristics of 22 children with SHOX anomalies and familial short stature suggestive of léri-weill dyschondrosteosis. Horm Res Paediatr. 2011;76(3):178-85. doi: 10.1159/000329359.

52. Ogata T, et al. Growth hormone and gonadotropin-releasing hormone analog therapy in haploinsufficiency of SHOX. Endocr J. 2001;48(3):317-22. doi: 10.1507/endocrj.48.317.

53. Munns CFJ, et al. Effect of 24 months of recombinant growth hormone on height and body proportions in SHOX haploinsufficiency. J Pediatr Endocrinol Metab. 2003;16(7):997-1004. doi: 10.1515/JPEM.2003.16.7.997.

54. Blum WF, et al. Height gains in response to growth hormone treatment to final height are similar in patients with SHOX deficiency and Turner syndrome. Horm Res. 2009;71(3):167-72. doi: 10.1159/000197874.

55. Gravholt CH, et al. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. Eur J Endocrinol. 2017;177(3):G1-G70. doi: 10.1530/EJE-17-0430.

56. Dantas NCB, et al. Adult height of patients with SHOX haploinsufficiency with or without GH therapy: a real-world single-center study. Horm Res Paediatr. 2022;95(3):264-74. doi: 10.1159/000524374.

57. Dantas NCB, et al. Adult height in 299 patients with turner syndrome with or without growth hormone therapy: results and literature review. Horm Res Paediatr. 2021;94(1-2):63-70. doi: 10.1159/000516869.

58. Montalbano A, et al. Retinoic acid catabolizing enzyme CYP26C1 is a genetic modifier in SHOX deficiency. EMBO Mol Med. 2016;8(12):1455-69. doi: 10.15252/emmm.201606623.

59. Scalco RC, et al. Effectiveness of the combined recombinant human growth hormone and gonadotropin-releasing hormone analog therapy in pubertal patients with short stature due to SHOX deficiency. J Clin Endocrinol Metab. 2010;95(1):328-32. doi: 10.1210/jc.2009-1577.

60. Blum WF, et al. GH treatment to final height produces similar height gains in patients with SHOX deficiency and Turner syndrome: results of a multicenter trial. J Clin Endocrinol Metab. 2013;98(8):E1383-92. doi: 10.1210/jc.2013-1222.

61. Bechtold S, et al. Pubertal height gain in Ullrich-Turner syndrome. J Pediatr Endocrinol Metab. 2006;19(8):987-93. doi: 10.1515/jpem.2006.19.8.987.

62. Morin A, et al. Adult height in Turner Syndrome girls after long-term growth hormone treatment. Medicina (B Aires). 2009;69(4):431-6.

63. Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24. doi: 10.1038/gim.2015.30.

APÊNDICES

APÊNDICE A - Critérios do ACMG/AMP para classificação das variantes alélicas quanto à patogenicidade

Critério muito forte para patogenicidade	
PVS1	Variante *nonsense, frameshift*, em sítios de splice canônicos ±1 ou 2, códon de iniciação ou deleção de 1 ou vários exons (*null variant*) em um gene no qual variantes com perda de função (*LoF*) são mecanismos conhecidos de doença
Critérios fortes para patogenicidade	
PS1	Troca de aminoácido já estabelecida como patogênica, independente da troca do nucleotídeo
PS2	Variante *de novo* em um paciente com doença sem história familiar (com paternidade confirmada)
PS3	Presença de estudos funcionais (*in vitro* ou *in vivo*) bem estabelecidos que suportem o efeito prejudicial no gene ou sua proteína
PS4	A prevalência da variante nos indivíduos afetados é significativamente maior quando comparada com a prevalência em controles
Critérios moderados para patogenicidade	
PM1	Variante localizada em uma região *hotspot* para mutações e/ou região importante já estabelecida com um domínio funcional da proteína, sem variantes descritas como benignas
PM2	Variante ausente nos bancos de dados populacionais (*gnomAD*, *ABraOM*) (ou presente em frequência extremamente baixa, para os casos recessivos)
PM3	Variante detectada em *trans* com uma variante patogênica para as doenças recessivas
PM4	Modificação no tamanho da proteína causada por deleções ou inserções *in-frame* em região não repetitiva ou por variantes que perdem o códon de parada (tipo *stop-loss*)

PM5	Nova variante *missense* em um aminoácido em que uma variante *missense* diferente já foi estabelecida como patogênica
PM6	Variante considerada de novo, sem confirmação da paternidade
Critérios moderados para patogenicidade	
PP1	Segregação da variante (em um gene conhecido como causador de doença) com a doença em uma família com vários membros afetados
PP2	Variante *missense* em um gene com uma baixa taxa de mutações *missense* benignas e no qual esse tipo de variante é considerado um mecanismo comum de doença
PP3	Várias ferramentas computacionais suportam a evidência de efeito deletério no gene ou proteína (conservação evolutiva, impacto no *splicing* etc)
PP4	O fenótipo do paciente ou a história da família são bastante específicos de uma doença monogênica
PP5	Variante recém-publicada como patogênica, porém o laboratório não apresenta condições de fazer um estudo funcional independente
Critérios fracos para benignidade	
BP1	Variante *missense* em um gene no qual variantes com perda de função (*LoF*) são mecanismos conhecidos de doença
BP2	Variante detectada em *trans* com uma variante patogênica dominante com penetrância completa ou observada em *cis* com uma variante patogênica em qualquer tipo de herança
BP3	Deleções ou inserções *in-frame* em região repetitiva sem função conhecida
BP4	Várias ferramentas computacionais sugerindo ausência de efeito deletério no gene ou proteína (conservação evolutiva, impacto no *splicing* etc)
BP5	Variante encontrada em um caso com uma base molecular alternativa para doença

BP6	Variante recém-publicada como benigna, porém o laboratório não apresenta condições de fazer um estudo funcional independente.
BP7	Variante sinônima cujas ferramentas de predição de *splicing* não predizem impacto na sequência consenso de *splice* nem a criação de um novo sítio, e o nucleotídeo não é conservado
Critérios fortes para benignidade	
BS1	A frequência do alelo é maior que a esperada para a doença
BS2	Variante para doença recessiva (em homozigose), dominante (em heterozigose) ou ligada ao X (hemizigose), com penetrância completa e de início precoce, observada em indivíduo adulto saudável
BS3	Presença de estudos funcionais (*in vitro* ou *in vivo*) bem estabelecidos mostrando ausência de efeito prejudicial para a proteína ou *splicing*
BS4	Ausência de segregação nos indivíduos afetados da família
Critério benigno por si só	
BA1	Frequência alélica maior que 5% nos bancos de dados populacionais (*1000Genomes*, ExAC)

PVS: critério muito forte para patogenicidade; PS: critério forte para patogenicidade; PM: critério moderado para patogenicidade; PP: critério fraco para patogenicidade; BA: critério benigno por si só; BS: critério forte para benignidade; BP: critério fraco para benignidade. Lof: perda de função (de loss of function); gnomAD: banco de dados: Genome Aggregation Database. ABraOM: banco de dados: Arquivo Brasileiro Online de Mutações.

Fonte: Adaptado de Richards e colaboradores, 2015[63].

APÊNDICE B – Normas para combinação dos critérios do ACMG/AMP para classificação das variantes alélicas identificadas

Classificação	Combinação dos critérios
Variante patogênica	(I) 1 critério muito forte para patogenicidade (PVS1) e (a) ≥ 1 critérios fortes para patogenicidade (PS1-PS4) ***ou*** (b) ≥ 2 critérios moderados para patogenicidade (PM1-PM6) ***ou*** (c) 1 critério moderado para patogenicidade (PM1-PM6) ***e*** 1 critério fraco para patogenicidade (PP1-PP5) ***ou*** (d) ≥ 2 PP (PP1-PP5) (II) ≥ 2 critérios fortes para patogenicidade (PS1-PS4) ***ou*** (III) 1 critério forte para patogenicidade (PS1-PS4) ***e*** (a) ≥ 3 critérios moderados para patogenicidade (PM1-PM6) ***ou*** (b) 2 critérios moderados para patogenicidade (PM1-PM6) ***e*** ≥ 2 critérios fracos para patogenicidade (PP1-PP5) ***ou*** (c) 1 critérios moderados para patogenicidade (PM1-PM6) ***e*** ≥ 4 critérios fracos para patogenicidade (PP1-PP5)

Variante provavelmente patogênica	(I) 1 critério muito forte para patogenicidade (PVS1) ***e*** 1 critério moderado para patogenicidade (PM1-PM6) ***ou*** (II) 1 critério forte para patogenicidade (PS1-PS4) ***e*** 1 ou 2 critérios moderado para patogenicidade (PM1-PM6) ***ou*** (III) 1 critério forte para patogenicidade (PS1-PS4) ***e*** ≥ 2 critérios fracos para patogenicidade (PP1-PP5) ***ou*** (IV) ≥ 3 critérios moderados para patogenicidade (PM1-PM6) ***ou*** (V) 2 critérios moderados para patogenicidade (PM1-PM6) ***e*** ≥ 2 critérios fracos para patogenicidade (PP1-PP5) ***ou*** (VI) 1 critério moderado para patogenicidade (PM1-PM6) ***e*** ≥ 4 critérios fracos para patogenicidade (PP1-PP5)
Variante benigna	(I) 1 critério benigno por si só (BA1) ***ou*** (II) ≥ 2 critérios fortes para benignidade (BS1-BS4)
Variante provavelmente benigna	(I) 1 critério forte para benignidade (BS1-BS4) ***e*** 1 critério fraco para benignidade (BP1-BP7) ***ou*** (II) ≥ 2 critérios fracos para benignidade (BP1-BP7)
Variante de significado incerto	(I) outra combinação de critérios não mencionada acima ou

(II) critérios para benignidade e patogenicidade contraditórios

PVS: critério muito forte para patogenicidade; PS: critério forte para patogenicidade; PM: critério moderado para patogenicidade; PP: critério fraco para patogenicidade; BP: critério fraco para benignidade; BS: critério forte para benignidade; BA: critério benigno por si só.
Fonte: Adaptado de Richards e colaboradores, 2015[63].

ANEXOS

ANEXO A **-** Contribuições científicas como co-autor durante o doutoramento

I. Schnöll C, Krepischi ACV, Renck AC, Amato LGL, Kulikowski LD, **Dantas NCB**, Costa EMF, Mendonca BB, Latronico AC, Jorge AAL, Silveira LFG. SIN3A defects associated with syndromic congenital hypogonadotropic hypogonadism: an overlap with Witteveen-Kolk syndrome. Neuroendocrinology. 2023;113(8):834-843. doi: 10.1159/000529615.

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SIN3A Defects Associated with Syndromic Congenital Hypogonadotropic Hypogonadism: An Overlap with Witteveen-Kolk Syndrome

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Keywords
Congenital hypogonadotropic hypogonadism · Kallmann syndrome · Gonadotropin-releasing hormone · Witteveen-Kolk syndrome · Endocrinology · Genetics · Molecular biology

Abstract
Introduction: Congenital hypogonadotropic hypogonadism (CHH) is a rare condition caused by GnRH deficiency. More than 40 genes have been associated with the pathogenesis of CHH, but most cases still remain without a molecular diagnosis. Mutations involving the same gene (e.g., *FGFR1, PROK2/PROKR2, CHD7*) were found to cause normosmic CHH and Kallmann syndrome (KS), with and without associated phenotypes, illustrating the coexistence of CHH with signs of other complex syndromes. The Witteveen-Kolk syndrome (WITKOS), caused by defects of the *SIN3A* gene, is a heterogeneous disorder characterized by distinctive facial features, microcephaly, short stature, delayed cognitive, and motor development. Although micropenis and cryptorchidism have been reported in this syndrome, WITKOS has not been formally associated with CHH so far. ***Patients and Methods:*** A man with KS associated with mild syndromic features (51) and a boy with global developmental delay, syndromic short stature, micropenis and cryptorchidism

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II. Rezende RC, Noronha RM, Keselman A, Quedas EPS, **Dantas NCB**, Andrade NLM, Bertola DR, Malaquias AC, Jorge AAL. Delayed Puberty Phenotype Observed in Noonan Syndrome Is More Pronounced in Girls than Boys. Horm Res Paediatr. 2022;95(1):51-61. doi: 10.1159/000522670.

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Delayed Puberty Phenotype Observed in Noonan Syndrome Is More Pronounced in Girls than Boys

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Keywords
Noonan syndrome · Pubertal delay · Pubertal development · Pubertal disorders

Abstract
Introduction: Pubertal delay is described as one of the clinical features in Noonan syndrome (NS) and it may be one of the factors causing short adult height in those patients. The present study aimed at characterizing pubertal development in NS and identifying pubertal delay predictors. ***Methods:*** We analyzed 133 individuals with a molecular diagnosis of NS and clinical puberty evaluation. We characterized delayed puberty as pubertal onset after 12 years in girls and 13.5 years in boys, according to parameters of the Brazilian population. To investigate its predictors, we correlated the age at onset of puberty with several characteristics and genotype in a multilevel regression model. For comprehending pubertal development in NS, we assessed age and anthropometric measures at each Tanner stage and adult age. ***Results:*** The mean age at puberty onset for girls was 11.9 ± 1.9 years and for boys, 12.5 ± 1.7 years, significantly later than the Brazilian population ($p = 0.025$; $p < 0.001$). Girls (49.1%) presented delayed puberty more frequently than boys (27.9%, $p = 0.031$). Body mass index standard deviation scores (SDS) and insulin growth factor 1 SDS at puberty onset significantly predicted later puberty entry. Height gain from the onset of puberty to adult height was lower in children with pubertal delay. ***Conclusion:*** Pubertal delay is characteristically found in children with NS, more frequently in females. The low weight of patients with NS could modulate the age of puberty, just as the increase in overweight/obesity in the general population has shown an effect on reducing the age of onset of puberty.

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III. Andrade NLM, Funari MFA, Malaquias AC, Collett-Solberg PF, Gomes NLRA, Scalco R, **Dantas NCB**, Rezende RC, Tiburcio AMFP, Souza MAR, Freire BL, Krepischi ACV, Longui CA, Lerario AM, Arnhold IJP, Jorge AAL, Vasques GA. Diagnostic yield of a multigene sequencing approach in children classified as idiopathic short stature. Endocr Connect. 2022;11(12):e220214. doi: 10.1530/EC-22-0214.

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RESEARCH

Diagnostic yield of a multigene sequencing approach in children classified as idiopathic short stature

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Abstract

Objective: Most children with short stature remain without an etiologic diagnosis after extensive clinical and laboratory evaluation and are classified as idiopathic short stature (ISS). This study aimed to determine the diagnostic yield of a multigene analysis in children classified as ISS.

Design and methods: We selected 102 children with ISS and performed the genetic analysis as part of the initial investigation. We developed customized targeted panel sequencing, including all genes already implicated in the isolated short-stature phenotype. Rare and deleterious single nucleotide or copy number variants were assessed by bioinformatic tools.

Results: We identified 20 heterozygous pathogenic (P) or likely pathogenic (LP) genetic variants in 17 of 102 patients (diagnostic yield = 16.7%). Three patients had more than one P/LP genetic alteration. Most of the findings were in genes associated with the growth plate differentiation: *IHH* ($n = 4$), *SHOX* ($n = 3$), *FGFR3* ($n = 2$), *NPR2* ($n = 2$), *ACAN* ($n = 2$), and *COL2A1* ($n = 1$) or involved in the RAS/MAPK pathway: *NF1* ($n = 2$), *PTPN11* ($n = 1$), *CBL* ($n = 1$), and *BRAF* ($n = 1$). None of these patients had clinical findings to guide a candidate gene approach. The diagnostic yield was higher among children with severe short stature (35% vs 12.2% for height SDS ≤ or > −3; $P = 0.034$). The genetic diagnosis had an impact on clinical management for four children.

Conclusion: A multigene sequencing approach can determine the genetic etiology of short stature in up to one in six children with ISS, removing the term idiopathic from their clinical classification.

Key Words
- idiopathic short stature
- multigene sequencing analysis
- genetic
- mutation

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Hormone resistance and short stature: A journey through the pathways of hormone signaling

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ABSTRACT

Hormone resistances have been described in association with growth disorders, the majority involving the growth hormone (GH)/insulin-like growth factor 1(IGF-1) axis or hormones with specific paracrine-autocrine actions in the growth plate. Defects in hormone receptors or in proteins involved in intracellular signal transduction (post-receptor defects) are the main mechanisms of hormone resistance leading to short stature. The characteristic phenotypes of each of these hormonal resistances are very distinct and bring with them important insights into the role of each hormone and its signaling pathway. In this review, we discuss the molecular and clinical aspects of the main hormone resistances associated with short stature in humans.

1. Introduction

The human growth process is determined by a multitude of complex events that mediate a coordinated action of cell proliferation and differentiation. The longitudinal growth, and determination of an individual's height, relies mainly on the growth of long bones, which in turn depends on the proper balance between proliferation and differentiation of chondrocytes in the epiphyseal growth plate. Any disturbances in this harmonious process may result in a slowdown in the growth rate and/or promote a premature growth arrest, resulting in short stature. One well-characterized mechanism causing growth disorders is hormonal resistance disrupting the physiological growth process.

Hormones are molecules secreted by cells which exert their physiological function through receptor binding and activation. They can circulate in body fluids and act far from its point of origin or be distributed by diffusion across cell membranes in a paracrine way. Hormone resistance is defined as an inability of target tissues to respond normally to a particular hormone. Defects in hormone receptors or in proteins involved in intracellular signal transduction (post-receptor defects) are the main mechanisms of hormone resistance. Several of these defects have been described in association with growth disorders (Table 1), the majority involving the growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis or hormones with specific paracrine-autocrine action in the growth plate (Fig. 1). The phenotypes are very distinct and bring with them important insights into the role of each hormone, its signaling pathway for normal growth and other physiological functions.

In the present review, we describe the clinical and molecular aspects of the main hormone resistances which lead to short stature as the central phenotype, highlighting the differences in additional clinical characteristics and molecular basis.

2. Hormone resistance involving the GH/IGF-1 axis

2.1. The GH/IGF-1 axis

The secretion of growth hormone (GH) from the anterior pituitary is

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Exome Sequencing Identifies Multiple Genetic Diagnoses in Children with Syndromic Growth Disorders

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ANEXO B **-** Prêmio de 3º Lugar na categoria melhores temas livres orais- Área Básica/translacional no 35º Congresso Brasileiro de Endocrinologia e Metabologia (CBAEM) 2022.

CBEM2022
35º CONGRESSO BRASILEIRO DE ENDOCRINOLOGIA E METABOLOGIA
3 a 7 de Setembro • São Paulo

Certificado

Certificamos que o trabalho

Identification of new genetic modifiers of the phenotype in SHOX haploinsufficiency

dos autores:

NAIARA CASTELO BRANCO DANTAS, MARIANA F. A. FUNARI, NATHALIA LIBERATOSCIOLI MENEZES DE ANDRADE, RAÍSSA CARNEIRO REZENDE, LAURANA DE POLLI CELLIN, ANTÔNIO MARCONDES LERARIO, MIRIAN YUMIE NISHI, BERENICE BILHARINHO DE MENDONÇA, ALEXANDER AUGUSTO DE LIMA JORGE

Foi premiado na categoria **Prêmio Melhores Temas Livres Orais - ÁREA BÁSICA/TRANSLACIONAL em 3º Lugar** apresentado na modalidade **Apresentação Oral** durante o 35º Congresso Brasileiro de Endocrinologia e Metabologia, ocorrido de 3 a 7 de setembro de 2022, no Transamerica Expo Center

São Paulo, 7 de setembro de 2022

Marise Lazzaretti Castro
Presidente do 35º CBEM

Paulo Augusto Carvalho Miranda
Presidente da Comissão Científica do 35º CBEM

Cesar Luiz Boguszewski
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