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**Maria Felicia Faienza, Mariangela Chiarito, Giacomina Brunetti & Gabriele D'Amato**

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REVIEW

# Growth plate gene involment and isolated short stature

Maria Felicia Faienza [1] · Mariangela Chiarito [1] · Giacomina Brunetti [2] · Gabriele D'Amato [3]

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## Abstract

**Purpose** Short stature is a common clinical presentation, thus it is widely accepted that it is a polygenic trait. However, genome wide association and next generation sequencing studies have recently challenged this view, suggesting that many of the children classified as idiopathic short stature could instead have monogenic defects. Linear growth is determined primarily by chondrogenesis at the growth plate. This process results from chondrocyte proliferation, hypertrophy, and extracellular matrix secretion, and it is perfectly coordinated by complex networks of local paracrine and endocrine factors. Alterations in genes which control growth plate development can explain a large number of cases of isolated short stature, allowing an etiological diagnosis.

**Methods/Results** We reviewed recent data on the genetic alterations in fundamental cellular processes, paracrine signaling, and cartilage matrix formation associated with impaired growth plate chondrogenesis. In particular we focused on growth plate gene involvement in nonsyndromic short stature.

**Conclusions** The identification of genetic basis of growth failure will have a significant impact on the care of children affected with short stature.

**Keywords** Growth plate · Isolated short stature · Gene · Children · SHOX · Aggrecan

## Introduction

Genetic variability is the main determinant of stature in humans, as the height is a highly heritable character.

The rapid development of technology has led to new discoveries in the genetic causes of syndromic and non-syndromic short stature. In the last decade, the next generation sequencing technologies have allowed an extensive use of "omic" assays in clinical practice. In particular, whole exome sequencing (WES), has been successfully employed for the discovery of gene variants as monogenic causes of growth disorders. Therefore, the number of nonsyndromic children who were classified as having idiopathic short stature (ISS), in the absence of laboratory data explaining growth impairment, has gradually decreased. Until now, more than 600 common variants (frequency > 1%) have been related to height [1]. However, genome wide association (GWA) studies suggested that the major effect on height variability is due to rare variants (frequency < 1%) in genes involved in growth plate development [2, 3]. It is possible that polymorphisms and mild mutations may modulate height within the normal range and cause slight short stature, whereas alterations with a greater effect on protein function, gene haploinsufficiency, or biallelic mutations may cause isolated monogenic short stature or skeletal dysplasia. Therefore, the term of "isolated" short stature has been introduced to point out monogenic conditions that explain the short stature phenotype in nonsyndromic children. In addition, the term of familial short stature (FSS) is commonly used to describe a growth disorder that is vertically transmitted. Consequently, a short child is classified as having FSS if at least one of the parents is also short.

✉ Maria Felicia Faienza
mariafelicia.faienza@uniba.it

[1] Paediatric Unit, Department of Biomedical Sciences and Human Oncology, University of Bari "Aldo Moro", Bari, Italy

[2] Department of Basic Medical Sciences, Neuroscience and Sense Organs, Section of Human Anatomy and Histology, University of Bari "A. Moro", Bari, Italy

[3] Neonatal Intensive Care Unit, Di Venere Hospital, Bari, Italy

## Genetic defects involved in growth failure

In the past, the failure of linear growth in childhood has been mostly related to genetic defects of growth

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hormone-insulin-like growth factor type 1 (GH–IGF1) axis. However, genetic defects in this axis are not common causes of short stature. In addition, other genes involved in the GH–IGF1 axis have recently been associated with monogenic short stature. Loss-of-function mutations in pregnancy-associated plasma protein-A2 (PAPP-A2) that cleaves insulin like growth factor binding protein (IGFBP)-3 and IGFBP-5, cause short stature by decreasing biologically active IGF1 [4]. Genetic variants which cause increased activity of Stanniocalcin 2 (STC2), that normally inhibits PAPP-A, would determine short stature by decreasing the amount of bioavailable IGF1 [5]. The genetic causes of growth failure can involve not only alterations in hormones, hormonal receptors, or relative pathways, but also defects in fundamental cellular processes (intracellular signaling pathways, transcriptional regulation, and DNA repair), extracellular matrix, or paracrine signaling. Mutations in genes involved in RAS-mitogen-activated protein kinase signaling pathway, such as *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *BRAF*, and *NRAS*, which are responsible for Noonan Syndrome and other Rasopathies, are also relatively common causes of isolated short stature [6]. In these patients, mild facial dysmorphisms and cardiac defects can be associated with short stature [7]. Heterozygous and/or mild mutations in short stature homeobox (*SHOX)*, natriuretic peptide receptor 2 (*NPR2)*, fibroblast growth factor receptor 3 (*FGFR3)*, Indian hedgehog (*IHH*) and aggrecan (*ACAN)* genes have been associated with isolated short stature [8], while homozygous and/or severe mutations in the same genes cause syndromic short stature with skeletal malformations [9]. A factor adding complexity is that heterogeneous mutations in one gene can result in different clinical entities, previously defined as separate conditions ("allelic heterogeneity"). On the other hand, a single clinical disorder can result from mutations in different genes ("locus heterogeneity"). Moreover, mutations of some genes not only affecting the development and/or function of the growth plate but also nonskeletal structures, result in associated congenital abnormalities (syndromic short stature) [3].

Here, we review growth plate gene involvement in nonsyndromic short stature. Specifically, we focus on the genetic alterations in fundamental cellular processes, paracrine signaling, and cartilage matrix formation associated with impaired growth plate chondrogenesis and therefore growth failure.

## Mechanisms of growth plate regulation

Linear growth is the result of chondrogenesis at growth plate, and it is regulated by a complex network of local and systemic factors [10]. The bone formation starts with pre-chondrogenic mesenchymal cells which differentiate into proliferative chondrocytes and into metabolically active hypertrophic chondrocytes (Fig. 1) [11]. During chondrocyte proliferation and hypertrophic differentiation, endoplasmic reticulum (ER) produces extracellular proteins that constitute structural components of the extracellular matrix (ECM) and trigger the differentiation in osteoblasts. Bone health also depends on the balance between the activity of osteoblasts, the bone-forming cells, and osteoclasts, the bone-reabsorbing cells, and this balance has been demonstrated to be impaired in many pediatric diseases [12]. This balance is regulated by several cytokines, and in particular by RANK/RANKL/osteoprotegerin and Wnt/β-catenin pathways which control osteoclastogenesis and osteoblastogenesis, respectively. The inhibitors of Wnt/β-catenin pathway, sclerostin and DKK-1, can impair bone remodeling both in inherited and acquired pediatric diseases [13, 14]. Furthermore, different cellular conditions, e.g., high-protein request during bone development or pathological conditions, can alter ER homeostasis and lead to the accumulation of poorly folded proteins. This pathological condition, called "ER stress", induces morphological and functional changes in chondrocytes, and activates an unfolded protein response, which represents an adaptive mechanism to restore ER homeostasis [15]. This complex quality control system blocks cellular protein synthesis, increases production of chaperones and other folding proteins, and leads to degradation of aggregated proteins. As a consequence of prolonged ER stress in chondrocytes, skeletal diseases, such as chondrodysplasias, can develop [15].

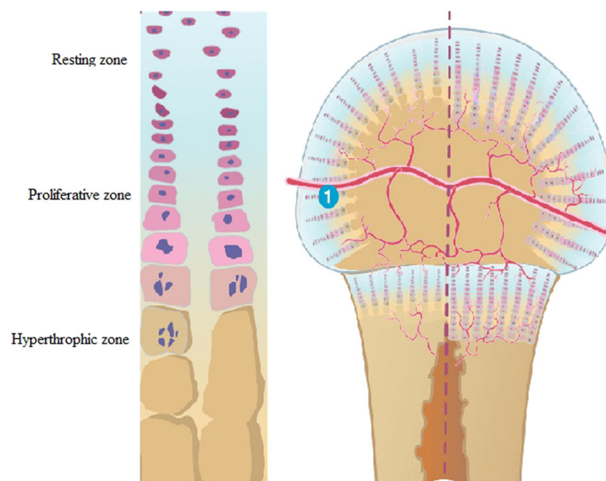


**Fig. 1** The structure of growth plate: resting zone, proliferative zone, and hypertrophic zone

The processes of chondrocyte differentiation, proliferation, cartilage matrix secretion, vascular, and bone cell invasion at the growth plate are regulated by intracellular signaling molecules, ECM proteins and paracrine factors. In addition, GH, IGF1, thyroid hormone, glucocorticoids, androgens, and estrogens control endochondral bone

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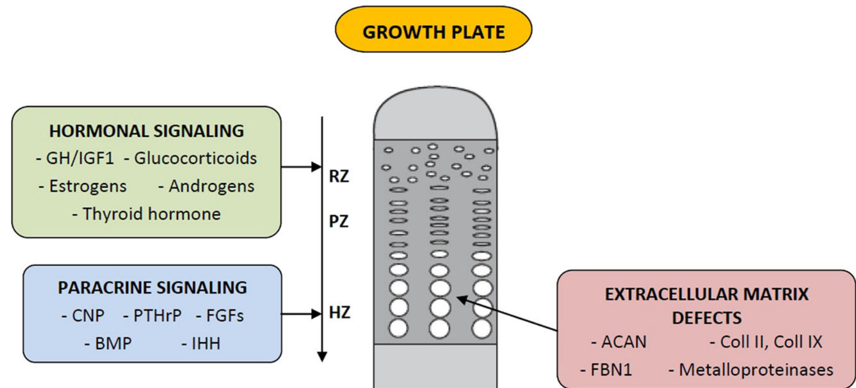


**Fig. 2** Mechanisms of growth plate regulation: hormonal signaling, paracrine signaling, and extracellular matrix defects. RZ resting zone, PZ proliferative zone, HZ hypertrophic zone, GH growth hormone, CNP C-type natriuretic peptide, PTHrP parathyroid hormone-related protein, FGFs fibroblast growth factors, BMP bone morphogenic protein, IHH Indian hedgehog

formation (Fig. 2). Alterations in genes involved in these signaling pathways impair bone growth, causing isolated short stature, or skeletal dysplasia. Furthermore, Guo et al. demonstrated that height variants are significantly enhanced in the regulatory regions of growth plate chondrocytes, and that the most of them resides in intergenic portions of the genome [16]. Using a genetic sequencing called "ATAC-seq", that allows epigenetic profiling of growth plate chondrocytes, the authors identified stretches of DNA acting as on/off switches for genes in human chondrocytes, showing that many of the small changes in DNA that contribute to differences in human height remain within these DNA switches [16]. These ATAC-seq peaks overlapping height GWAS variants are enriched for nearby differentially expressed growth plate genes. Thus, this motif analysis revealed that some of these human height variants may alter transcription factors and gene regulatory processes with important roles in chondrogenesis [16].

## Alterations in genes involved in fundamental cellular processes

Loss- or gain-of-function mutations of genes involved in fundamental cellular processes result in isolated short stature or skeletal dysplasia. The main mechanisms controlling cellular functions include transcriptional factors, intracellular pathways, DNA repair, and cell proliferation. Among the transcriptional factors, the *SHOX* is responsible of isolated short stature (Table 1).

### SHOX gene

The *SHOX* gene is located in the pseudoautosomal region of both sex chromosomes, and it is expressed in growth cartilage, especially in hypertrophic chondrocytes [17]. The role of the *SHOX* gene as regulator of the growth plate has to be yet fully elucidated. It stimulates and coordinates chondrocytes proliferation and differentiation by increasing natriuretic peptide B (*NPPB)*, and inhibiting *FGFR3* gene expression. Furthermore, *SHOX* interacts with the SOX trio (*SOX9*, *SOX5*, and *SOX6* genes), which play an important role in cartilage matrix synthesis (Fig. 3) [18]. *SHOX* alterations cause a broad phenotypic spectrum of short stature, ranging from isolated short stature to skeletal dysplasias. Homozygous *SHOX* mutations cause the *Langer mesomelic dysplasia*, which is characterized by severe short stature, shortening of the long bones (mesomelia), and Madelung deformity [19]. Heterozygous *SHOX* mutations produce a milder skeletal dysplasia, the *Léri-Weill dyschondrosteosis* [20], or are responsible of 2.6–12% of ISS [17, 21]. Furthermore, *SHOX* deficiency contributes to the short stature and skeletal abnormalities in Turner syndrome [22]. The most of *SHOX* defects are deletions involving the gene or regulatory regions, while the remaining are point mutations. No genotype–phenotype correlation has been identified in individuals with *SHOX* alterations. However, deletion in downstream *SHOX* enhancer has been associated with a milder phenotype [17]. Particular attention should be given to the regulatory regions of *SHOX* gene, as deletions located outside the coding region are more commonly associated with short stature without other specific findings. The degree of short stature is variable, ranging from a severe growth impairment to a height within the normal range. Abnormal body proportion, defined by the sitting height/height ratio for age and sex (SH/H SDS) > 2, is the main feature in children with isolated short stature caused by *SHOX* haploinsufficiency [23]. An algorithmic approach to screen subjects with isolated short stature for *SHOX* defects has been proposed [24]. Minor abnormalities found in subjects carrying *SHOX* deficiency are shortening of the fourth and fifth metacarpals, high-arched palate, increased angle of the elbow, scoliosis, and micrognathia.

## Genetic defects affecting paracrine regulation of growth plate

Paracrine factors are key regulators of endochondral ossification and defects in genes that encode for these factors

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**Table 1** Growth plate gene involvement in nonsyndromic short stature

| Gene | Frequency (%) | Function | Gene alterations | Clinical phenotype |
|---|---|---|---|---|
| *SHOX* | 2.6–12 | Regulation of chondrocytes proliferation and cartilage matrix synthesis | Heterozygous deletions/point mutations | Short stature with disproportionate body proportions |
| *CNP* | 1.8–13.6 | Regulation of endochondral ossification | Heterozygous loss-of-function mutations | Short stature with abnormal body proportions, small hands phenotype |
| *IHH* | 3.4 | Regulation of chondrocyte proliferation and hypertrophy | Heterozygous mutations | Short stature, variable brachydactyly, shortening of the middle phalanx of the fifth finger |
| *ACAN* | 1.4 | Major proteoglycan component in the extracellular matrix of the growth plate | Heterozygous mutations | Proportionate short stature, accelerated bone age, mild midface hypoplasia, brachydactyly, flat nasal bridge, early-onset osteoarthritis |

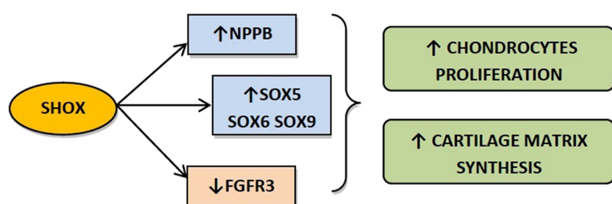


**Fig. 3** The role of the *SHOX* gene as a regulator of the growth plate. NPPB natriuretic peptide B, FGFR3 fibroblast growth factor receptor 3

have been associated with a small percentage of ISS (up to 2%), and many cases of FSS (Fig. 2). GWA studies identified 78 genes encoding for paracrine regulators of growth plate, such as C-type natriuretic peptide (CNP), IHH, parathyroid hormone-related protein (PTHrP), bone morphogenetic protein (BMP)/TGF-β superfamily signaling, and FGFR3 signaling [25]. The alterations of CNP and IHH have recently been associated with isolated short stature or FSS.

## CNP

CNP and its principal receptor, natriuretic peptide receptor B (NPR-B), are expressed in the hypertrophic zone of the growth plate and are the main regulators of the endochondral ossification, by stimulating chondrocytes and synthesis of cartilage matrix [26, 27]. CNP binding to the NPR-B receptor induces cyclic guanosine monophosphate synthesis, and activates an intracellular signaling cascade that involves the inhibition of the FGFR3 pathway [28, 29].

Homozygous loss-of-function mutations of the gene encoding for CNP receptor (*NPR2*) cause acromesomelic dysplasia type Maroteaux [30], while heterozygous *NPR2* mutations are associated with isolated short stature (Table 1) [31, 32]. Until now, the prevalence of heterozygous *NPR2* mutations in familial cases of isolated short stature has ranged from 1.8 to 13.6% [33]. Recently, heterozygous mutation of gene encoding for CNP have been identified in two Brazilian and four Spanish families with isolated short stature, with a height SDS ranging from −4.3 to −2.3 and small hands phenotype [34]. Similar to *SHOX* haploinsufficiency, the carriers of *NPR2* mutation have a variable degree of short stature, with height at the lower limit of the normal range. Abnormal body proportion (SH/H SDS > 2) and nonspecific skeletal alterations, as short metacarpals, have been also observed among subjects with *NPR2* mutations. Due to the phenotypic heterogeneity, there are not criteria to select patients with short stature for *NPR2* molecular screening.

## FGFR3

FGFR3 signaling has an important role in several cellular processes, including proliferation, differentiation, and cell survival. In proliferating chondrocytes FGFR3 pathway is activated by FGF9 and FGF18, which are expressed in the perichondrium and adjacent mesenchyme [35, 36]. Activation of FGFR3 stimulates STAT1, MAPK, and PP2a that trigger downstream signals (p107, p21Waf1/Cip1, Sox9), suppressing chondrocyte proliferation, and regulating matrix production and chondrocyte differentiation [37]. CNP expression has been proven to attenuate the phenotype of achondroplasia in mice through inhibition of MAPK signaling, which restored matrix production and hypertrophic differentiation [38]. FGFR3 gene mutations cause several forms of disproportionate short stature, of which the least severe phenotype is hypochondroplasia. Recently, alterations in FGFR3 gene have been associated with proportionate short stature, especially when transmitted in an autosomal dominant manner [39]. However, the pathogenic significance of these variants has not been demonstrating yet. Studies of WES demonstrated that single-gene variants are frequent among families with severe FSS, with a prevalence of those affecting the growth plate [40]. In particular, 90% of children examined by WES showed at least one genetic variant with potential clinical significance in genes which affect growth. A single-gene variant was found in 52% of cases, with the most prevalence in genes involved

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in growth plate disorders (*COL2A1*, *COL11A1*, *ACAN*, *FGFR3, FLNB*, and *IGF1R*), and less frequently in genes encoding for IGF-proteins (*IGFALS* and *HMGA2*), and related to Noonan syndrome (*PTPN11* and *SOS1*) [40]. The results of this study demonstrated that genetic variants controlling growth plate play an important role in children affected with severe FSS.

### IHH

PTHrP has been demonstrated to regulate chondrocyte differentiation in the fetal murine growth plate [41, 42]. The secreted protein IHH, expressed in murine hypertrophic chondrocytes, is in turn a key regulator of PTHrP by a local negative feedback loop [42]. In fetal bone, IHH stimulates periarticular cartilage to express PTHrP, which then acts on the prehypertrophic chondrocytes to inhibit further differentiation [42]. Experimental data demonstrated that IHH and PTHrP are expressed mainly in early hypertrophic chondrocytes in the human growth plate. The levels of expression of IHH and PTHrP are higher in early stages of puberty than later, suggesting that they may be involved in the regulation of pubertal growth in humans [43].

In humans, heterozygous mutations in *IHH* gene determine short stature with variable brachydactyly and shortening of the middle phalanx of the fifth finger, and craniosynostosis (Table 1) [44]. However, *IHH* gene mutations have been associated with short stature with broad skeletal findings in some families, with an autosomal dominant inheritance pattern [45]. For these children, an improvement of stature has been observed after growth hormone treatment [45].

## Genetic defects affecting cartilage extracellular matrix

Growth plate chondrocytes produce an ECM enrich in collagens and proteoglycans, which provide support and interacts with paracrine signaling molecules regulating chondrocyte proliferation and differentiation. Therefore, mutations in genes that encode matrix collagens, proteoglycans, noncollagenous proteins cause growth failure with a wide phenotypic spectrum.

### *ACAN* gene

Aggrecan, encoded by *ACAN* gene, is the major proteoglycan component in the ECM of the growth plate, thus having a key structural and functional role [46]. The ECM has a critical role in the structural support of chondrocytes, and also by acting as a medium in which signaling molecules and growth factors are able to spread through the avascular cartilage toward the target cells [47]. The ECM is mainly composed of collagen, proteoglycans, hyaluronan, and link proteins with smaller quantities of other specific proteins of the matrix [48]. The sulfated glycosaminoglycans (GAGs), attached to the main protein of the aggrecan, produce a large negatively charged molecule that allows the hydration of the cartilage tissue, as well as the binding of growth factors and morphogens crucial for the maturation and function of the chondrocytes [47]. The sulfated GAG chains attached to the aggrecan are essential in regulating and modifying normal bone growth [47]. Early hypertrophic chondrocyte maturation, and early invasion of blood vessels and osteoblasts in the growth plate could explain the advanced bone age and the premature epiphyseal fusion in patients with *ACAN* alterations [49]. Initially, *ACAN* mutations were associated with two rare skeletal dysplasias associated with severe osteoarthritis [50]. In 2014, heterozygous *ACAN* mutations were identified as cause of FSS in children showing accelerated bone maturation, early-onset osteoarthritis, and no skeletal findings (Table 1) [51]. Up to now, more than one hundred subjects belonging to families with autosomal dominant inherited short stature have been identified as carriers of *ACAN* mutations [51–55]. Children carrying *ACAN* alterations would seem to have growth impairment before birth, and *ACAN* mutations have been reported in short children born small for gestational age who would benefit from GH treatment associated with 2 years of gonadotropin-releasing hormone analog [56].

The most of children with *ACAN* mutations have proportionate short stature with advanced bone age, reduced pubertal spurt followed by early growth arrest, low adult height and body disproportion. Other features are brachydactyly, mild midface hypoplasia, and flat nasal bridge. Some individuals present early-onset osteoarthritis, with a variable degree of severity [57].

## Conclusions

The identification of genetic basis of growth failure will have a significant impact on the care of children affected with short stature. The known genetic defects explain 25–40% of children with ISS indicating that many of genetic causes of nonsyndromic short stature remain to be discovered. Genetic variants responsible for development and function of the growth plate play an important role, especially in children born SGA.

Further research should be performed to better characterize the growth plate gene involvement and to evaluate the gene-specific GH responsiveness to better individualize the management of short stature.

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**Author contributions** M.F.F. designed the study and prepared the first draft of the paper. She is guarantor. M.C. and G.B. design the figures and revised critically the paper; G.D. search the literature data and revised the final draft. All authors revised the paper critically for intellectual content and approved the final version. All authors agree to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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