A new era of genetic diagnosis for short stature children
A review

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ABSTRACT
Children with short stature are often presented to pediatric endocrinologists. Short stature is defined as the height that is more than two standard deviations below the corresponding mean height for a specific age and sex in a reference population. Endocrine dysfunctions, including growth hormone deficiency/insensitivity, hypothyroidism, cortisol excess, precocious puberty, chronic disease (renal disease, diabetes mellitus, or inflammatory disease), and genetic disorders, should be assessed in patients presenting with short stature. In addition to medical history, physical examination, endocrine study, skeletal survey, and genetic testing are important for identifying the cause of short stature. Based on the next-generation sequencing analysis in patients with short stature, different genes that are unrelated to syndromic or non-syndromic short stature were identified. In particular, the genetic causes of short stature disrupting the growth plates and the pituitary-insulin-like growth factor axis have expanded. In recent years, the molecular level of chondrogenesis in the growth plates, including paracrine signals, extracellular matrix, and fundamental intracellular signals, has been reported. Moreover, new insights into the molecular pathogenesis of short stature are emerging. This article aimed to review the genetic causes of primary growth impairment in idiopathic short stature conditions.

Keywords: Chondrogenesis; Genetic testing; Growth; Growth plate; High-throughput nucleotide sequencing

INTRODUCTION
Short stature is defined as the height that is less than the third percentile or two standard deviations (SD) of the mean height for a specific age, sex, and race [1]. The gain of height during childhood results from normal growth plate chondrogenesis [2]. Normal growth is affected by many factors, including endocrine signals (growth hormone [GH], thyroxine, glucocorticoids, and sex hormones), inflammatory cytokines, nutrition, and extracellular fluid. Therefore, growth velocity during infancy, childhood, and adolescences is considered an important indicator of health...
status in pediatrics [3,4]. Short stature is caused by reduced growth plate chondrogenesis, which can be a result of primary, secondary, or idiopathic impairment of the growth plate [2-4]. Previously, to explain short stature in children, we evaluated distinct parameters such as systemic inflammatory disease, hypothyroidism, GH deficiency, and idiopathic short stature [1,4]. However, normal results for thyroid function, inflammatory markers, liver function, renal function, GH provocation test, and karyotyping have shown that these tests are not sufficient to explain short stature in children [3-6]. In genetic testing of short stature, karyotyping was conducted for Turner syndrome in 1960. Short stature homeobox (SHOX) gene, which is the causative gene for Léri-Weill syndrome, and methylation disorder of the insulin-like growth factor 2 (IGF2) gene, leading to Silver-Russell syndrome, were identified around 2000 [6]. Chromosome microarray can detect copy number variants of specific genes. Moreover, next-generation sequencing (NGS) methods have been introduced. We consider these new methods for determining the cause of short stature when patients show severe GH deficiency, multiple pituitary hormone deficiencies, and GH insensitivity [7]. In addition, conditions such as small gestational age (SGA) without catch-up growth, accompanying congenital anomalies, dysmorphic face, developmental delay or intellectual disability, or microcephaly have been indicated for genetic testing [7-10]. Profound short stature (height < -3.0 SD), even in isolated short stature (ISS) conditions, should be considered a monogenic disease [8-10]. Mild-type autosomal dominant (AD) skeletal dysplasia can be underdiagnosed in familial short stature (FSS) conditions [11,12]. Recently, several genes involved in the endochondral ossification process and their frequency in ISS conditions have been identified. Furthermore, several reports have demonstrated that mutations in the same genes, such as SHOX, natriuretic peptide receptor B (NPR2), aggrecan (ACAN), and fibroblast growth factor receptor 3 (FGFR3) can cause a wide spectrum of phenotypic abnormalities, ranging from skeletal dysplasia to ISS [13,14].

This review describes the genetic causes of primary growth plate dysfunction that leads to short stature in children.

**LINEAR GROWTH AND GROWTH PLATE**

The growth plate is a thin structure of cartilage found near the ends of the long bones and in the vertebrae. It consists of three zones: the resting zone, proliferative zone, and hypertrophic zone [15]. Linear growth results from the regulation of growth plate chondrogenesis with normal hormone signaling interactions and its growth plate status [15-17]. Primary growth plate dysfunction is classified by disorders of the paracrine, cartilage extracellular matrix (ECM), and intracellular signaling pathways [16-18]. It can lead to short stature or malformed bone (skeletal dysplasia) or short bone without malformation (ISS).

**Paracrine disorder**

Different autocrine and paracrine factors are secreted by the growth plate chondrocytes, which act locally on other chondrocytes [15]. Genetic defects in these autocrine or paracrine factors, their receptors, or other involving signaling can impair normal growth plate chondrogenesis and growth in children [15,18]. The paracrine signaling pathway includes IGFs, C-type natriuretic peptide (CNP), fibroblast growth factors (FGFs), parathyroid hormone-related protein (PThrP), Indian hedgehog (IHH), bone morphogenetic protein (BMP) signaling, and Wnt signaling pathway components (Table 1) [15,18].

IGF2 is a growth factor that plays a role in fetal and postnatal growth [19]. IGF2, located on chromosome 11p15.5, is an imprinted gene, and hypomethylation of paternal IGF2 leads to Silver-Russell syndrome (MIM#180860) [19]. In addition, pleomorphic adenoma gene 1 (PLAG1) on human chromosome 8q12, and high-mobility group AT-hook 2 (HMGA2) on chromosome 12q14 have been reported as upstream regulators of IGF2 [20].

CNP acts on the NPR2 and stimulates growth plate chondrogenesis, including chondrocyte differentiation, hypertrophy, and matrix synthesis [21]. Mutations in either the ligand (CNP) or the receptor (NPR2) impair linear growth. Biallelic mutations of NPR2 at chromosome 9p13 cause acromesomelic dysplasia type Maroteaux (chondrodysplasia with short stature, MIM#602875), whereas monoallelic loss-of-function NPR2 mutations lead to ISS [21,22]. NPR2 mutations are responsible for 2% to 6% of patients with ISS [23,24]. Natriuretic peptide C (NPPC) gene at chromosome 2q37 encodes CNP. Individuals with monoallelic mutation of NPPC were reported with short stature with a tendency towards the development of small hands [25]. CNP analogues are the first approved precision medicine to enhance bone growth in achondroplasia caused by gain-of-function mutations in FGFR3 [14]. FGFR signaling acts through FGFR3 which negatively regulates growth plate chondrogenesis [14]. The clinical phenotypes of gain-of-function mutations in FGFR3 include thanatophoric dysplasia, achondroplasia, and hypochondroplasia [14].

PThrP and IHH signaling make a local negative feedback loop in the growth plate that controls chondrogenesis includ-
### Table 1. Gene (locus) lists and disorders associated with primary growth plate dysfunction

<table>
<thead>
<tr>
<th>Paracrine signal</th>
<th>Gene (locus)</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin-like growth factor (IGF2)</td>
<td>H19/IGF2 (pat transmission) IGF2 (pat) (11p15.5) CDKN1C (mat) (11p15.5) PLAG1 (8q12), HMGA2 (12q14)</td>
<td>Silver-Russell syndrome (SRS)</td>
</tr>
<tr>
<td>C-type natriuretic peptide (CNP)</td>
<td>NPR2 (9p13.3)</td>
<td>Isolated short stature (autosomal dominant, AD) Epiphyseal chondrodysplasia, Miura type (AD) Acromesomelic dysplasia 1, Maroteaux type (autosomal recessive, AR)</td>
</tr>
<tr>
<td>Fibroblast growth factor (FGF)</td>
<td>FGFR3 (4p16.3)</td>
<td>Achondroplasia or hypochondroplasia (AD) Thanatophoric dysplasia I, II (AD) Idiopathic short stature (AD)</td>
</tr>
<tr>
<td>Parathyroid hormone-related protein (PTHrP) and Indian hedgehog (IHH) signaling</td>
<td>PTH1R (3p21.31) IHH (2q35)</td>
<td>Blomstrand lethal chondrodysplasia (AR) Jansen metaphyseal chondrodysplasia (AD) Brachydactyly type A1 (AD) Short stature without skeletal abnormalities (AD)</td>
</tr>
<tr>
<td>Bone morphogenic protein (BMP) signaling</td>
<td>BMP1B (4q22.3)</td>
<td>Acromesomelic dysplasia 3 (AR) Brachydactyly, type A1, D (AD) Brachydactyly, type A2 (AD)</td>
</tr>
<tr>
<td>WNT signaling</td>
<td>ROR2 (9q22.31) NXX (17p13.3) WNT5A (3p14.3) DVL1 (1p36.33) DVL3 (3q27.1)</td>
<td>Robinow syndrome, AR1 Robinow syndrome, AR2 Robinow syndrome, AD1 Robinow syndrome, AD2 Robinow syndrome, AD3</td>
</tr>
<tr>
<td>Extracellular matrix synthesis: collagen matrix protein</td>
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<tr>
<td>Type II collagen</td>
<td>COL2A1 (12q13)</td>
<td>Achondrogenesis, type II, hypochondrogenesis (AD) Spondyloepiphysialdysplasia (AD) Spondyloepimetaphysialdysplasia (AD)</td>
</tr>
<tr>
<td>Type IX collagen</td>
<td>COL9A1 (6q13) COL9A2 (1p34.2) COL9A3 (20q13.33)</td>
<td>Multiple epiphyseal dysplasia (MED), type 6 (AD) MED2 (AD) MED3, with or without myopathy (AD)</td>
</tr>
<tr>
<td>Type X collagen</td>
<td>COL10A1 (6q22.1)</td>
<td>Metaphyseal chondrodysplasia, Schmid type (AD)</td>
</tr>
<tr>
<td>Type XI collagen</td>
<td>COL11A1 (1p21.1) COL11A2 (6p21.32)</td>
<td>Marshall syndrome (AD), Otspondyloepimetaepiphysialdysplasia (AD, AR)</td>
</tr>
<tr>
<td>Extracellular matrix synthesis: non-collagen matrix protein</td>
<td></td>
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<tr>
<td>Aggrecan</td>
<td>ACAN (15q26.1)</td>
<td>Short stature and advanced bone age, with or without early onset osteoarthritis and/or osteochondritis dissecans (AD) Spondyloepiphysialdysplasia, Kimberley type (AD) Spondyloepimetaphysialdysplasia, aggrecan type (AR)</td>
</tr>
<tr>
<td>Cartilage oligomeric matrix protein (COMP)</td>
<td>COMP (19p13.11)</td>
<td>MED1 (AD) Pseudoachondroplasia (AD)</td>
</tr>
<tr>
<td>Matrilin-3 (MATN3)</td>
<td>MATN3 (2p24.1)</td>
<td>MEDS (AD) Spondyloepimetaphysialdysplasia, Borochowitz-Cormier-Daire type (AR)</td>
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<tr>
<td>Fibrillin 1</td>
<td>FBN1 (15q21.1)</td>
<td>Acromicric dysplasia trait (AD), Geleophysic dysplasia 2 (AD)</td>
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ing proliferation and hypertrophy of chondrocyte [17]. PTHrP actions through parathyroid hormone 1 receptor (PTH1R). Both biallelic (loss-of-function) and monoallelic (gain-of-function) mutations in PTH1R at chromosome 3p21.31 impair growth plate chondrogenesis [26].

Blomstrand lethal chondrodysplasia (MIM#215045) is an autosomal recessive (AR) disorder of PTH1R mutations characterized by profound dwarfism with short limbs, fetal hydrops, prenatal lethality, facial dysmorphism, and advanced skeletal maturation [26].

Jansen metaphyseal chondrodysplasia (MIM#156400) is an AD disorder of PTH1R mutations characterized by profound dwarfism with short limb and hypercalcemia [26]. IHH is a main regulator of osteoblast and chondrocyte differentiation during endochondral ossification [16,17]. It directly affects chondrocyte proliferation and stimulates PTHrP synthesis [16,17]. Heterozygous mutations in IHH at chromosome 2q35 showed two phenotypic features: brachydactyly type A1 (MIM#112500) or ISS. The ISS phenotype due to IHH mutation includes conditions of mild disproportionate short stature with short and dysplastic middle phalanx of the 5th finger [27].

BMP signaling is an evolutionarily highly conserved process that controls critical pathway of not only bone and cartilage formation but also all organ system during embryogenesis
and development [28,29]. Genetic defects in the transforming growth factor β superfamily, including several BMPs and growth differentiation factors, are associated with skeletal dysplasia characterized by brachydactyly [28,29]. BMP type-1 receptor (BMPR1B) is a receptor for growth differentiation factor 5 (GDF5) which is essential for bone development and homeostasis [28]. Biallelic loss-of-function mutations of BMPR1B lead to acromesomelic dysplasia 3, whereas monoallelic mutations cause brachydactyly type A1 or A2 [28,29].

Robinow syndrome is a rare genetic skeletal dysplasia syndrome characterized by short stature with short limbs, typical facial anomalies, and genitourinary or heart anomaly [30]. This disorder is caused by disruption of the Wnt signaling pathway through mutations in receptor tyrosine kinase-like orphan receptor 2 (ROR2), Wnt family member 5A (WNT5A; the ligand of ROR2), or dishevelled segment polarity protein 1 (DVL1) genes [16,18,30]. The ROR2 is fundamental for the normal development of the bone, cardiac, and reproductive system [30].

Extracellular matrix synthesis
The ECM produced by chondrocytes is the non-cellular component present within every tissue and organ [2,16,17]. It consists with collagens and proteoglycans and networks with paracrine signal pathway leading to chondrocyte differentiation and proliferation [2,16,17].

The disorder of collagen formation (collagenopathies) in the growth plate are caused by mutations of genes for collagen types II, IX, X, and XI (Table 1) [11,15,31]. The type II collagen is major components of cartilage, and its disorders are AD conditions caused by collagen type II alpha 1 chain (COL2A1) gene mutations with complete penetrance [2]. The clinical characteristics includes disproportionate short stature, skeletal dysplasia, palate anomaly, hearing disorder, and ocular anomalies, but the ranges are various [2,15]. Type IX collagen acts as a link that connects type II collagen with other cartilage components and networks with cartilage oligomeric matrix protein (COMP) and matrilin-3 (MATN3), which are essential components of the ECM [2,15]. Mutations in genes (COL9A1, COL9A2, and COL9A3) encoding alpha 1–3 chains of type IX collagen can cause multiple epiphyseal dysplasia (MED) characterized by mild short stature, radiologic epiphyseal hypoplasia and/or irregularity, and early onset of osteoarthritis involving the hip and knee joints [2,15]. Type X collagen disorder is a metaphyseal chondrodysplasia. Also known as Schmid type, this is caused by heterozygous mutations in COL11A1. Type XI collagen disorder is caused by mutations in COL11A1 (Stickler dysplasia type 2 with ocular alterations) or COL11A2 (ostospondylopseudopemphyseal dysplasia) [15,31,32].

Defects in the synthesis of non-collagen matrix proteins, including ACAN, COMP, MATN3, and fibrillin 1 (FBN1), could lead to impaired growth plate chondrogenesis (Table 1) [12,15,18]. ACAN is major proteoglycan in articular and growth plate cartilage and is necessary for its construction and function [12,16,18]. Diseases associated with ACAN mutations at chromosome 15q26.1 include short stature and advanced bone age with or without early onset osteoarthritis and/or osteochondritis dissecans (MIM#165800, AD trait), spondyloepimetaphyseal dysplasia, Kimberley type (MIM#608361, AD trait), and spondyloepimetaphyseal dysplasia, ACAN type (MIM#612813, AR trait) [12,16]. Midfacial hypoplasia, broad thumb and great toe or advanced bone age were characteristic features in ISS due to ACAN and ACAN mutations are responsible for 1.4% of patients with ISS [33].

COMP is an essential component of the ECM which is necessary for the normal cartilage formation and its conversion to bone [18,34]. COMP mutation at chromosome 19p13.11 causes two skeletal dysplasias: pseudoachondroplasia (MIM#177170) and dominant MED (MIM#132400) [18,34]. Mutations in MATN3 located on chromosome 2p24.1 are responsible for dominant MED (MIM#607078) [18,35]. Mutations in the FBN1 gene considered as causative gene for Marfan syndrome are also found to cause geleophysic dysplasia (MIM#614185) and acromicric dysplasia (MIM#102370) [18,35].

Fundamental cellular disorder
Fundamental cellular disorders are a complex group of short stature syndromes (Table 1). These fundamental cellular processes affect all cells and not just chondrogenesis. These disorders include defects in SHOX, RASopathies, GNAS inactivation, 3M syndrome, DNA damage repair syndromes, and primordial dwarfism [2,18].

The SHOX gene is in the pseudoautosomal region of both sex chromosomes and is a critical transcription factor for the chondrocyte function [4]. It stimulates chondrocyte proliferation and differentiation through enhancing CNP and inhibiting FGFR3 expression [16]. Biallelic mutations in SHOX cause Langer mesomelic dysplasia (MIM#249700), whereas monoallelic mutations in SHOX lead to various phenotypes such as Léri-Weill dyschondrostosis [MIM#127300] showing Madelung deformity or ISS [16,17]. SHOX gene mutations are responsible for 1.1% to 22.2% of ISS [13].

The RASopathies are a group of diseases caused by genetic defects involving the Ras/mitogen-activated protein kinase
(MAPK) signaling pathway including Noonan syndrome, Noonan syndrome with multiple lentigines (MIM#151100), Costello syndrome (MIM#218040), cardiofaciocutaneous syndrome (MIM#115150), neurofibromatosis type 1 (MIM#162200), and Legius syndrome (MIM#611431) [17,36,37]. The Ras/MAPK signaling pathway integrates growth signals from including GH, CNS, FGFs, and epidermal growth factor [17,37].

The 3M syndrome (MIM#273750) is a rare AR disorder that has been named after the initials of the three investigators, Miller, McKusick, and Malvaux, who first described it in 1975 [38,39]. Clinical manifestations include prenatal/postnatal growth restriction, characteristic face, relative macrocephaly, and normal endocrine function and intelligence [6,39,40]. Three causative genes, cullin 7 (CUL7), obscurin-like protein 1 (OBSL1), and coiled-coil domain-containing protein 8 (CCDC8), were identified for 3M syndrome, with prevalence rates of 77.5%, 16.3%, and less than 5%, respectively. CUL7 is an ubiquitin ligase that controls microtubule integrity, and OBSL1 is a cytoskeletal adaptor linking which controls a microtubule stabilizer, and OBSL1 and CCDC8 make complex with CUL7 [38]. Loss-of-function in 3M complex leads to abnormal microtubule and genome integrity and growth failure [38].

DNA damage repair syndrome, including Fanconi anemia and Bloom syndrome, should be distinguished in short stature individuals who were born SGA [6]. These disorders are rare but are not treated with GH treatment due to increased cancer risk. Their clinical characteristics are pre- and postnatal growth failure and microcephaly [6,18]. Fanconi anemia results in changes in skin pigmentation, including café-au-lait spots, deformity in the upper limb, thumb, or radius, and other hematologic abnormalities such as anemia, thrombocytopenia, or neutropenia [41]. Even though the FA complementation group A (FANCA) gene is responsible for 60% to 70% of Fanconi syndrome cases, more than 20 genes have been reported to cause Fanconi anemia. Thus, a multi-gene panel will help identify the causative gene [41] in such cases. Bloom syndrome shows infantile-onset sun-sensitive facial rash, feeding intolerance, and immunoglobulin abnormalities [42]. The BLM gene is the only known gene for Bloom’s syndrome [42].

Primordial dwarfism, including Seckel syndrome, microcephalic osteodysplastic primordial dwarfism, and Meier-Gorlin syndrome is associated with cell division and fundamental cellular processes and showed profound prenatal and postnatal short stature and microcephaly [2,18]. Defects in several genes contribute to these disorders.

**INDICATION OF EXOME SEQUENCING IN SHORT STATURE**

For genetic testing in short stature, the 2021 clinical practice resource of the American College of Medical Genetics and Genomics recommended that clinical exome sequencing should be considered in patient not only with endocrine dysfunction (severe GH deficiency or multiple pituitary hormone deficiency) but also with profound short stature (height <-3 SD), SGA without catch-up growth, FSS (one parent height <-2.0 SD), parental consanguinity, or additional clinical features including intellectual disability, microcephaly, skeletal abnormalities, or facial dysmorphism [9]. Previously, NGS panel for syndromic short stature identified the genetic causes between 16.5% and 46% [9]. Recently, NGS target for short stature have been expanded to ISS and FSS, and diagnostic yield of NGS for ISS was between 16.5% and 33.3%, and FSS group treated with GH therapy showed 52% of detection rate [12,43].

**CONCLUSION**

GH supplementation is a well-known treatment for not only enhancing adult height in children with short stature but also improving body proportion in Prader-Willi syndrome [44-46]. However, the response to GH is inconsistent with that of the underlying disease. The evaluation of genetic causes, including growth plate dysfunction, is important to predict the responsiveness to GH or clinical prognosis in children with short stature. Exome sequencing aids in determining an accurate diagnosis in patients with unknown growth disorders. It also helps in determining the pathogenesis of abnormal growth, thereby providing insight into human physiology and genetics. A carefully selected cohort is important to enhance the diagnostic yield of exome sequencing.

In conclusion, exome sequencing has expanded the area of growth failure and changed the traditional approach employed to determine the cause of short stature in individuals. It has enabled us to not only understand the molecular pathophysiology but also investigate patients for ISS or FSS considered. Both these conditions are implicated in growth plate dysfunction. It must be taken into consideration that ISS may no longer be idiopathic, and FSS may not be benign. The exome sequencing can be useful in identifying the molecular defects of growth plate dysfunction, including paracrine signaling defects, ECM defects, and other fundamental cellular processes. Identification of the causative genetic defect will enable the prediction of individuals who would be good responders or contraindi-
Short stature and primary growth plate dysfunction is indicated for GH therapy. It would also aid in close follow-up to anticipate complications in the early treatment of short stature.

CONFLICTS OF INTEREST
No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS
Conception or design: YMK.
Acquisition, analysis, or interpretation of data: YMK.
Drafting the work or revising: YMK.
Final approval of the manuscript: YMK.

REFERENCES
21. Rintz E, Wegrzyn G, Fujii T, Tomatsu S. Molecular mechanism of induction of bone growth by the C-type natriuret-