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Clinical and Genetical Approach to Skeletal Dysplasia

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INTRODUCTION

Skeletal dysplasias comprise of more than 200 diseases and are not infrequent as a whole. However, each entity of skeletal dysplasias is rather rare and many pediatricians are unfamiliar with the recent advances in understanding of the pathogenesis and the medical management of these diseases. Skeletal dysplasias are clinically important, especially for neonatologist since the respiratory complications sometimes result in neonatal death. Recent advances in the research on bone and cartilage metabolism have shed light on the molecular mechanisms of skeletal diseases and made a great contribution to the management of patients with skeletal dysplasia.[1-3] The identified genes responsible for skeletal dysplastic diseases are increasing in number (Table. 63.1).[4-8] Importantly, several new techniques have developed for diagnosis and treatment of patients with skeletal dysplasias.

Bone formation or skeletogenesis consists of two different pathways, membranous bone formation and enchondral bone formation. The former process is direct bone formation from mesenchymal cells without a cartilage mold. The skull is the representative bone formed via membranous bone formation. In contrast, most bones including long bones and vertebrae are formed by enchondral bone formation. Several important factors have been identified to be involved in this process and some of them are reviewed in this chapter.[1-7] Moreover, the defects of these factors lead to skeletal dysplastic diseases.

The manifestation of patients with skeletal dysplasia is various. In utero, polyhydroamnios and growth retardation of fetus is often associated with skeletal dysplasias. After birth, respiratory difficulty is the primary issue to be solved in patients with severe skeletal dysplasias. Characteristic faces and disproportionate short stature are often the hallmarks of skeletal dysplasias in childhood. Extraskeletal features including hypoplastic hair, skin legions and immunological abnormality imply specific skeletal dysplasia.

The wide variation in phenotypic severity is one of features of skeletal dysplasia. Sometimes the names of diseases are distinct to describe the difference in phenotype even when the same gene is responsible for the diseases. Such diseases are allelic and have similar features, although the severity and the survival rate are quite different. For example, mutations of the FGF receptor 3 (FGFR 3) gene are associated not only with achondroplasia, the most common chondrodysplasia, but also with thanatophoric dysplasia and hypochondroplasia.[9] Another example is the case of the defects in the type II collagen gene including hypochondrogenesis, spondyloepiphyseal dysplasia, Kniest and Stickler diseases.[10]

Table 63.1: Skeletal dysplasia and its responsible gene

1. FGF receptor or FGF	
Achondroplasia	FGFR3 (G1138A,C)
Hypochondroplasia	FGFR3 (C1659A,G; C1620A)
Thanatophoric dysplasia	FGFR3 (I: C742T, II: A1948G)
Pfeiffer syndrome	FGFR1 (C755G); FGFR2 (T1036C; G1037A)
Crouzon syndrome	FGFR2 (G1037A; T1036C,A; C1073G, etc)
Jackson-Weiss syndrome	FGFR2
Apert syndrome	FGFR2 (C934G; C937G)
Beare-Stevenson cutis gyrata syndrome	FGFR2
Muenke craniosynostosis	FGFR3 (C749G)
Hypophosphatemic rickets, AD	FGF23
2. Collagen	
Osteogenesis imperfecta	COL1A1; COL1A2
Achondrogenesis type II	COL2A1
Hypochondrogenesis	COL2A1
Spondyloepiphyseal dysplasia	COL2A1
Kniest dysplasia	COL2A1
Stickler dysplasia	COL2A1; COL11A2
Stickler dysplasia type2	COL11A1
Spondyloepimetaphyseal dysplasia (Strudwick type)	COL2A1
Multiple epiphyseal dysplasia type2	COL9A2
Metaphyseal dysplasia (Schmid)	COL10A1
3. Matrix protein	
Pseudoachondroplasia	COMP
Multiple epiphyseal dysplasia	COMP
Shprintzen-Goldberg syndrome	FBN1
Keutel syndrome	MGP
Multiple epiphyseal dysplasia	MATN3
4. Hormone, Hormone receptor, Signal transducer	
Metaphyseal dysplasia (Jansen)	PTH/PTHrP receptor (gain)
Blomstrand chondrodysplasia	PTH/PTHrP receptor (loss)
Albright osteodystrophy	Gsa
McCune-Albright syndrome	Gsa
Vitamin D dependency type I	1alpha-hydroxylase
Vitamin D dependency type II	Vitamin D receptor
Osteoporosis-pseudoglioma	LRP5 (loss)
High bone mass	LRP5 (gain)
Familial expansile osteolysis	TNFRSF11A
Juvenile Paget's disease	OPG
5. Enzyme	
Hypophosphatasia	TNS Alkaline phosphatase
Osteopetrosis	Carbonic anhydrase II, TCIGR1, CLCN7, GL
Pycnodysostosis	Cathepsin K
Smith-Lemli-Opitz syndrome	Δ^7-sterol reductase
Mucopolysaccharidoses (Hurler, Morquio, —)	lysosome enzyme
X-linked recessive chondrodydplasia punctata	arylsulfatase E
Rhizomelic chondrodysplasia punctata	PEX7
Coffin-Lowry syndrome	RSK-2
Noonan syndrome	PTPN11
SEMD Pakistani type	PAPSS2

Contd...

Contd...

Osteolysis Torg type	MMP2
Wolcott-Rallison syndrome	EIF2AK3
OLEDAID	NEMO
6. Transcription factors	
Boston-type craniosynostosis	MSX2
Campomelic dysplasia	SOX9
Cleidocranial dysplasia	CBFA1
Saethre-Chotzen syndrome	TWIST
Polysyndactyly type II	HOXD-13
Greig syndrome	GLI3
Polydactyly type A	GLI3
Ulnar-mammary syndrome	TBX3
Holt-Oram syndrome	TBX5
Townes-Brocks syndrome	SALL1
Nail-patella syndrome	LMX1B
Tricho-rhino-phalangeal dysplasia type1	TRPS1
Ellis-van Creveld	EVC
EEC syndrome	p63
Trichodentooseeous syndrome	DLX3
Leri-Weill dyschondrostosis	SHOX
7. Others	
Diastrophic dysplasia	sulphate transporter (DTDST)
Achondrogenesis type IB	sulphate transporter (DTDST)
Atelosteogenesis type 2	sulphate transporter (DTDST)
Craniometaphseal dysplasia	pyrophosphate transporter (ANK)
Chondrodysplasia	growth/differentiation factor-5
(Hunter-Thompson, Grebe) (CDMP-1)	
osteosclerosis	SOST
Brachydactyly type C	CDMP-1
Camurati-Engelmann	TGFB1
SED tarda	SEDL (ER-Golgi transport)
Cartilage-hair hypoplasia	RMRP
Kenny-Caffey syndrome	TBCE (chaperone protein)
Multiple synostosis syndrome	Noggin
Spondylocostal dysostosis	DLL3
Brachydacyly A1	IHH
Osteoporosis-pseudoglioma syndrome	LRP5
Progressive pseudorheumatoid dysplasia	WISP3
Brachydacyly B2	ROR2
Dyggve-Melchior-Clausen dysplasia	FLJ90130
Shwachman-Diamond	SBDS
(common mutations are described in parenthesis)	

In general, phenotype is severe in patients with skeletal dysplasias found in perinatal period, and long survival is not achieved in many of them mainly due to respiratory problems. Elucidation of the relationship between the skeletogenesis and lung development may lead to better treatments for severe forms of skeletal dysplasias. In contrast, the treatments for childhood cases of skeletal dysplasias have been improved such as growth hormone for achondroplasia, bisphosphonate for osteogenesis imperfecta and bone marrow transplantation for malignant osteopetrosis. Thus, the opprotunity has increased for pediatricians to treat patients with skeletal dysplasia.

In this manuscript, we take an overview of some of key players in the enchondral bone formation and their implication in skeletal diseases. We aim to help clinicians to manage patients with skeletal dysplasia with better understanding of the diseases. At first, molecular mechanisms of the proliferation and differentiation of chondrocytes in growth plate are reviewed. Then, recent advances in genetics and clinical aspects are described in several representative skeletal dysplastic diseases. Clinical approach to skeletal dysplasia is mentioned in the last part of the manuscript.

DEVELOPMENT OF CARTILAGE AND BONE

Enchondral bone formation is a multistep process where chondrocytes proliferate and differentiate step by step, resulting in layered structure, namely resting, proliferating, hypertrophic and calcifying zones (Fig. 63.1).[3] After the extracellular matrix is calcified, bone

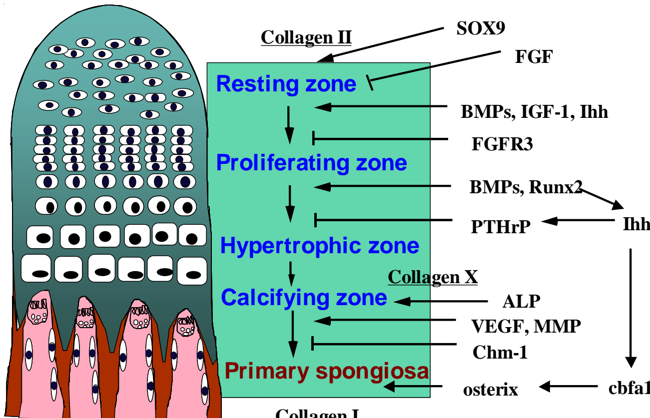


Fig. 63.1: Enchondral bone formation: Chondrocytes form the layer structure according to the stage of differentiation in growth plate and eventually cartilage is replaced by bone matrix, primary spongiosa. In the process of the enchondral bone formation, chondrocytes proliferate and differentiate into hypertrophic chondrocytes, then matrix is calcified. The main matrix proteins which are underlined are changed from collagen type II to collagen type I via collagen type X. Many molecules including SOX9, PTHrP and FGFR3 have been found to play an important role in the process as shown in the Figure. FGF: fibroblast growth factor, BMP: bone morphogenic protein, IGF-1: insulin-like growth factor, Ihh: Indian hedgehog, FGFR3: FGF receptor 3, PTHrP: PTH-related peptide, ALP: alkaline phosphatase, VEGF: vascular endothelial growth factor, MMP: matrix metaroproteinase, Chm-1: chondromodulin-1

tissue (primary spongiosa) replaces cartilage. During the chondrocytic differentiation, the main components of the matrix are changed from collagen type II to collagen type I via collagen type X. Recently, many molecules including SOX9, FGF receptor 3 (FGFR3) and parathyroid hormone-related peptide (PTHrP) have been found to play important roles in the process.[1-3] These integrated networks of such bioactive factors tightly control enchondral bone development in the limb. Moreover, the genes encoding these molecules are responsible for several types of skeletal dysplasias. The process of chondrocytic differentiation is terminated by the death of hypertrophic chondrocytes, followed by blood vessel invasion, which promotes replacement of the cartilaginous matrix by the primary spongiosa.

The first step of chondrocytic differentiation or chondrogenesis is the mesenchymal cell condensation of undifferentiated progenitor cells. The differentiation of mesenchymal precursor cells into chondrocytes requires a transcription factor, Sox9.[11,12] Sox9 is a member of the Sox family of transcription factors that contain high-mobility-group (HMG)-box DNA-binding domain. Sox9 up-regulates the expression of characteristic cartilaginous matrix genes such as collagens type II, IX, and XI, and aggrecan. In humans, haploinsufficiency of the Sox9 gene causes campomelic dysplasia (MIM 114290), which is characterized by deformities of long bones.[13] Most affected neonates die from respiratory failure due to hypoplastic tracheal and rib cartilage. Interestingly, some XY patients with campomelic dysplasia exhibit male-to-female sex reversal because Sox9 is involved not only in chondrogenesis but also in mammalian sex determination as a downstream regulator of the transcription factor, SRY.

FGFR3, as the other FGFRs, is a membrane bound protein, consisting of three immunoglobulin-like domains in the extracellular portions, a transmembrane domain and two tyrosine kinase domains in the intracellular portion.[14] FGFR3 is expressed in the proliferating chondrocytes. Its ligands, most importantly FGF18, bind to the FGFR3 and activate the receptor by its autophosphorylation

and the phosphorylation of other substrate proteins.[15] The major signaling pathways of FGFR3 are thought to be the Mitogen activating protein kinase (MAPK) pathway and the STAT-1 pathway. These signals mediate the inhibitory effects of FGFs on the proliferation and differentiation of chondrocytes.

The importance of FGF signaling in skeletal development was firstly revealed with the discovery that a point mutation in the transmembrane domain of FGFR3 causes achondroplasia (MIM 100800).[14] The abnormality of the FGFR3 gene is also associated with hypochondroplasia (MIM 146000), thanotophoric dysplasia (MIM 187600), and severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) syndrome.[16]

PTHrP was initially discovered as a causal factor of humoral hypercalcemia of malignancy.[17] However, gene-targeting experiments has revealed that PTHrP plays an important role in chondrogenesis via a local action.[18] PTHrP-null mice, for example, exhibit a severe disorder of enchondral bone formation, characterized by shortened bones and premature maturation and ossification of the cartilaginous tissues.[19] Mice lacking the parathyroid hormone (PTH)/PTHrP receptor exhibit the similar phenotypes. PTHrP and PTH, the latter of which is one of the main regulators of calcium and bone metabolism share the PTH/PTHrP receptor. Therefore, the PTH/PTHrP receptor mediates both the endocrine actions of PTH and the auto/paracrine actions of PTHrP. The PTH/PTHrP receptor is mainly expressed in the transitional zone between proliferating and hypertrophic cells (i.e., the pre-hypertrophic zone) in growth plate. PTHrP regulates the transition from proliferation to differentiation in chondrocytes.

Indian hedgehog (Ihh), a member of a family of proteins that are important for embryonic patterning, is highly expressed in the pre-hypertrophic zone and in the upper hypertrophic chondrocytes.[20, 21] Actually, Ihh is secreted by pre-hypertrophic and early hypertrophic chondrocytes. The mice Ihh-/- show abnormal chondrocytic differentiation and a failure to specify appendicular perichondrial cells to the osteoblast lineage.[22] Ihh induces the expression of PTHrP and inhibits differentiation of hypertrophic chondrocytes followed by the delay of the mineralization of cartilage matrix. This effect of Ihh on chondrocytic differentiation is mediated by increasing PTHrP production in the growth plate. Because PTHrP keeps chondrocytes in the proliferative pool and suppresses Ihh production, PTHrP and Ihh establish a negative-feedback loop that maintains a balance of chondrocyte proliferation and differentiation.[22] Ihh is also a potent stimulator of chondrocyte proliferation, and conversely an inhibitor of chondrocyte maturation.[23] In this sense, Ihh has the positive effect on normal chondrocyte proliferation in a PTHrP-independent manner. Heterozygous missense mutations in the Ihh gene have been recently identified as a cause of autosomal dominant form of brachydactyly type A1 (MIM 112500)(24). This type of brachydactyly is characterized by the shortening or the absence of middle phalanges.

Runx2, also called cbfa1, belongs to the Runt transcription factor family.[25-27] Runx2 was initially discovered as an essential molecule for osteoblast differentiation.[25-27] It has been shown that inactivating mutations of one allele of the human Runx2 gene cause cleidocranial dysplasia (MIM 119600).[25] However, further experiments revealed that Runx2 is also expressed in chondrocytes, and severe delays of chondrocyte maturation is found in genetically manipulated mice lacking this factor.[28]

Hypertrophic chondrocytes are the most characteristic in shape and produce the specific matrix protein, collagen type X. Then, the matrix is calcified and replaced by bone tissue. In other words, an avascular cartilage template is reconstructed into highly vascularized bone tissue. In contrast to immature chondrocytes which secrete angiogenic inhibitors, hypertrophic chondrocytes produce angiogenic stimulators such as vascular endothelial growth factor (VEGF) and FGF, and become a target for capillary invasion.[29] The invasion of blood vessels is critical in the last stage of chondrocyte maturation. The mammalian growth plate is highly hypoxic and

expresses the transcription factor hypoxia-inducible factor 1 (HIF-1), which belongs to the PAS subfamily of bHLH (basic helix-loop-helix) transcription factors and is known to induce the VEGF expression in many tissues and cells.[30] HIF-1 is not sufficient to induce VEGF expression in cartilage or other factor(s) blocks the inducible activity of HIF-1 before the maturation of chondrocytes becomes complete.

The degradation of calcified cartilage matrix is another important event for enchondral bone formation. During hypertrophic change, chondrocytes synthesize type X collagen, which is associated with denaturation and loss of type II collagen resulting from increased collagenase activity. The transcription factor Cbfa1 has been shown to regulate expression of matrix metalloproteinase (MMP) 13 (collagenase-3).[31] MMP-9 is also important enzyme for matrix degradation.[32] Remarkably, inactivation of matrix metalloproteinase-9 (MMP-9) resulted in bone phenotype similar to that found in VEGF-knockout mouse, suggesting the involvement of proteinases as well as VEGF in the processes of vascular invasion in growth plate and endochondral ossification. Indian hedgehog plays an important role in skeletal angiogenesis and calcification of cartilage.[33]

As described in this section, enchondral bone formation is elaborately regulated by many factors such as transcription factors, growth factors, enzymes and matrix proteins. Moreover, the defect in the regulation leads to dysplastic bone and cartilage formation both in human and mice.

CLINICAL AND GENETICAL ASPECTS OF SKELETAL DYSPLASIAS

Skeletal Dysplasias Associated with FGFR Abnormality

The abnormality of FGFR3 gene is associated with achondroplasia, hypochondroplasia, thanatophoric dysplasia and the most rare form, SADDAN.[14-16] Its cognate ligand remains unclear, but FGF18, 17 and 8 are likely to elicit its signal via binding to the FGFR3.[15] These ligands are produced in the perichondrium. The FGF family of proteins consists of at least 23 structurally related polypeptides that play a critical role in a variety of biological processes. The FGFRs represent a family of four tyrosine kinase receptors (FGFR1–4) that bind FGFs with variable affinity.

Achondroplasia is the most common form of rhizomeric dwarfism with a rate of one per 15000-30000 births. The disease is inheritated in an autosomal dominant manner, but more than 90% of the cases are sporadic. Advanced paternal age is reported in sporadic cases. Its clinical features are short stature caused by rhizomelic shortening of the limbs, characteristic faces with frontal bossing and mid-face hypoplasia, exaggerated lumbar lordosis, and trident hand. As typical radiologic features, caudal narrowing of the interpediculate distance, rather than the normal caudal widening, and a notchlike sacroiliac groove are found in children with achondroplasia. Interestingly, the same mutation of the FGFR3 gene is the cause of achondroplasia in almost all patients (G to A transition at nucleotide 1138).[34,35] This mutation causes the substitution of amino acid 380 (Gly380Arg), and can be recognized by PCR followed by digestion with restriction enzyme SfcI. The same mutation of FGFR3 was also found in Japanese patients with achondroplasia. The detection of this mutation is especially helpful in the diagnosis of neonatal patients with this disease. Other minor mutations are also reported (G to C at nucleotide 1138 and G to T at nucleotide 1123).

In our experience, some patients with achondroplasia are not recognized prenatally even when routine examination with ultrasonography is undertaken. This is partly because the disproportion develops more prominently after birth. In our study in which the relationship between age and clinical data (height, arm span and measurements of skeletal radiographs) were statistically analyzed in 27 achondroplasia patients with the G380R genotype, the height standard deviation score decreased with age. In making a clinical diagnosis of achondroplasia in early infancy, it should be noted that short stature and squared pelvis deformity are not prominent in some cases.[36]

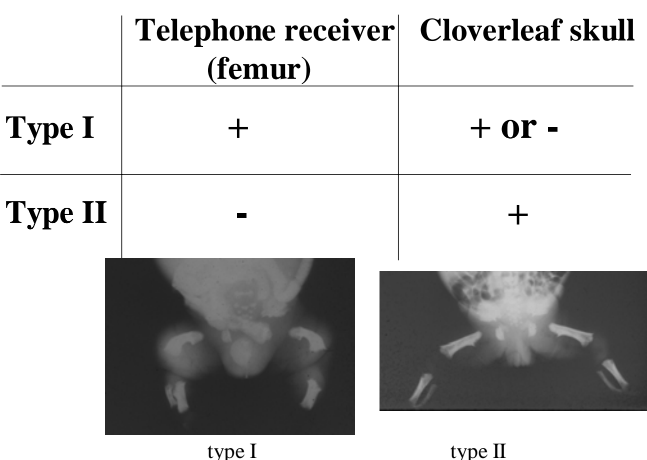
To treat the short stature of patients with achondroplasia, recombinant human growth hormone (GH) has been administered. First-year response is typically a 2-3 cm increase in growth velocity in prepubertal children.[37] However, the effect of GH treatment on the final adult height is not conclusive so far. GH treatment, at least in the prepubertal period, seems to influence degree of disproportion. The activating mutation of FGFR3 promotes apoptosis of chondrocytes. Insulin-like growth factor (IGF)-I, which is a mediator of GH, may reduce apoptosis of chondrocytes expressing mutated FGFR3.[38] More recently, it has been reported that c-type natriuretic peptide is effective to prevent shortening of long bones in the mice model for achondroplasia.[39]

Achondroplasia can be associated with respiratory difficulty in early infancy and childhood, although the degree of the difficulty is usually not so severe. One of the respiratory difficulties is manifested as sleep apnea syndrome.[40, 41] Although a large scale study on sleep apnea syndrome in achondroplasia is lacking, but approximately 30% patients with achondroplasia suffer from sleep anpea syndrome. In serious situation, sleep apnea syndrome leads to hypoxia, pulmonary hypertension, heart failure and sudden death. The causes of sleep apnea syndrome in achondroplasia are both obstruction of air way and failure of central control of respiration. The obstructive sleep apnea is derived from the small oral cavity with a relatively large tongue, hypertrophy of tonsilla and adenoid as well as weakness of trachea. Neurological problems including hydrocephalus and compression of the spinal cord due to a small foramen magnum are relatively common in achondrolasia and may cause central sleep apnea syndrome. Polysomnography is used to make a diagnosis of sleep apnea syndrome. Symptomatic patients require tonsillectomy, adenoidectomy or continuous positive airway pressure therapy.

Thanatophoric dysplasia, a lethal form of dwarfism, is also caused by mutations of the FGFR3 gene. In histological analyses, the most characteristic feature in thanatophoric dysplasia is the protrusion of perichondrium into the growth plate. The growth plate is irregular and narrow, as in other chondrodysplasias. An anomaly of the brain (abnormal fissure in the temporal lobe) is also observed. Thanatophoric dysplasia is divided into two subtypes according to the finding of the shape of the femur (I: curved femur, II: straight femur) (Fig. 63.2). A cloverleaf-like deformity of the skull is often associated with type II but may also be present in type I. The most common mutations in type I and II of thanatophoric dysplasia were reported to be Arg248Cys and Lys650Glu, respectively.

Hypochondroplasia is a relatively mild disease, manifested as short stature. The head is not affected. The spinal canal narrows in its caudal portion as is true in achondroplasia. Fingers are short, although hands are not of the trident type. Asn540Leu is reported to be the most common mutation found in this disease.

The other form in this family is called SADDAN, which stands for severe achondroplasia with developmental delay and acanthosis nigricans. The patients with SADDAN survive infancy without prolonged life-support measures. The FGFR3



	Telephone receiver (femur)	Cloverleaf skull
Type I	+	+ or -
Type II	-	+

Fig. 63.2: Two types of thanatophoric dysplasia: Thanatophoric dysplasia type 1 and 2 are characterized by telephone receiver-like bended femur and straight femur, respectively as x-ray figures are shown in the bottom. Cloverleaf skull is often associated with type 2 and sometimes with type1.

mutation (A1949T, Lys650Met) occurs at the nucleotide adjacent to the TD type II (TD2) mutation (A1948G: Lys650Glu) and results in a different amino acid substitution in the kinase domain activation loop.

All these disorders are autosomally dominant, mainly sporadic, and are caused by gain-of-function mutations, leading to a constitutively active FGFR3. The degree of activation of FGFR3 correlates well with the severity of the chondrodysplasia; the mutated FGFR3 found in thanatophoric dysplasia is the most activated form. This relationship provides the first evidence for negative regulation of chondrogenesis by FGFR3. Several animal models have established that FGFR3 negatively regulates chondrocyte proliferation. MAPK is a major downstream effecter of FGFR3 activity. Indeed, chondrocytes expressing a constitutively active form of MEK, the MAPK activator, present an achondroplasia phenotype similar to that observed with constitutively active FGFR3. Conversely, blockade of MAPK activation rescues achondroplasia obtained with constitutively active FGFR3. In addition, it has been shown that FGFR3 represses chondrocyte proliferation through activation of the transcription factor STAT1 by a yet unknown mechanism.

Collagenopathy

Type I collagen is the most abundant protein in bone. This type of collagen is synthesized as the heterotrimer of procollagens a1(I) and a2(I). Heterozygous mutations in the two genes for procollagen a1(I) and a2(I). (*COL1A1* and *COL1A2*) have been found in patients with osteogenesis imperfecta (MIM 166200, 166210, 166220, 166230).[42, 43] These mutations cause a change either in the structure of the protein or in the number of collagen molecules made. The severe forms of osteogenesis imperfecta are reported to have a glycine substitution within the Gly-X-Y amino acid repeat. Mutated collagens interfere with the formation of collagen fibrils. In contrast, null mutations of *COL1A1* cause the mild form of osteogenesis imperfecta.[44]

Osteogenesis imperfecta is characterized by low bone mineral density with fragility, and frequent bone fracture leads to deformity of long bones and chronic bone pain. It was classified into four forms by Sillence et al.[45]: a dominant form with blue sclerae, type I; a perinatally lethal OI syndrome, type II; a progressively deforming form with normal sclerae, type III; and a dominant form with normal sclerae, type IV. However, Type IV is rather heterogeneous and includes patients with a moderate to severe disease who do not fit types I or III. Thus, other types of OI (V, VI, and VII) without identifiable collagen type I mutations have been recently described.[43]

There is no curative treatment for osteogenesis imperfecta. However, the therapy using pamidronate, a derivative of bisphosphonate, has recently developed and successfully alleviated symptoms in children with OI. In short, cyclic intravenous administration of pamidronate improved bone mineral density and reduced the frequency of bone fracture. However, there are few studies reporting the outcome measures of bisphosphonate therapy in children with OI. Glorieux et al. observed that in patients with severe osteogenesis imperfecta aged 3 to 16 years, intravenous pamidronate (mean annual dose 6.8 mg/kg) increased lumbar vertebral bone mineral density (BMD) by an average of 42% per year.[46] The same group also demonstrated that in children with OI types III or IV less than 2 years of age, a 12-month treatment with pamidronate at a mean dose of 12.4 mg/kg increased lumbar vertebral BMD by 86 to 227%. Recently, Astrom and Soderhall reported that monthly infusions of pamidronate for 2 to 9 years resulted in gradual increase in total body and lumbar spine bone density in children with OI.[47]

As another novel treatment for patients with severe osteogenesis imperfecta, transplantation of bone marrow cells containing adult mesenchymal stem cells has been attempted.[48] Mesenchymal stem cells can differentiate into multiple cell types present in several tissues, including bone, fat, cartilage, and muscle, making them ideal candidates for cell-based therapies. More recently, mesenchymal cell therapy using isolated mesenchymal stem cells has been reported.[49] In the report, donor marrow-derived

mesenchymal cells were administrated to treat six children who had undergone standard bone marrow transplantation for severe osteogenesis imperfecta. Five of six patients showed engraftment in one or more sites, including bone, skin, and marrow stroma, and had an acceleration of growth velocity during the first 6 months post-infusion.

Adult stem cells can be used after the ***ex vivo*** genetic manipulation to correct genetical disorders. For osteogenesis imperfecta, one study in which adeno-associated viral vectors were used to disrupt dominant-negative mutant *COL1A1* collagen genes in mesenchymal stem cells from patients with a severe form of osteogenesis imperfecta was reported.[50] It seems that the gene was successfully targeted in adult human stem cells.

Collagen type II, IX, X and XI are expressed in the cartilaginous tissue. Collagen type II specific to cartilage matrix is synthesized as the homotrimer of collagen *a*1(II). Mutations in the single gene for type II procollagen have been found in patients with type II achondrogenesis (MIM 200610)-hypochondrogenesis, spondyloepiphyseal dysplasia (MIM 183900), Kniest dysplasia (MIM 156550) and Stickler (hereditary arthro-ophthalmopathy) syndrome (MIM 108300).[51] The platyspondylic lethal skeletal dysplasias (PLSD), Torrance type (MIM 151210) was found to be caused by *COL2A1* mutations.[52] The platyspondylic lethal skeletal dysplasias is a heterogeneous group of chondrodysplasias characterized by severe platyspondyly and shortening of limbs. Interestingly, the most common form of PLSD is thanatophoric dysplasia (TD), which is caused by mutations in the *FGFR3* as described previously.

Type X collagen is a homotrimeric, short-chain, nonfibrillar extracellular matrix component. The expression of type X collagen is restricted to hypertrophic chondrocytes. The mutation in the *COL10A1* is associated with Schmid metaphyseal chondrodysplasia (MIM 156500).[53] It is reported that Stickler syndrome type 2 with a different vitreoretinal phenotype from that of type 1 is caused by a mutation in the *COL11A1* (MIM 604841). Multiple epiphyseal dysplasia (MIM 132400) is caused by a mutation in the *COL9A1* or the *COMP* gene.[54, 55] The *COMP* gene is also responsible for pseucoachondroplasia (MIM 177170), which is one of the most frequent skeletal dysplasias and resembles achondroplasia except for head and face.[55]

Other Chondrodysplasias

The chondrodysplastic disease, achondrogenesis is characterized by severely impaired chondrogenesis with extremely short limbs and narrow thorax, leading to the perinatal death of patients with this disease. Fetal hydrops and hydroamnios are also associated with achondrogenesis. Although achondrogenesis has been subtyped in several ways, the definition reported by Borochowitz et al., based on detailed analyses of clinical, radiological and morphological studies, seems to have received consensus: type I (MIM 200600) A (Houston-Harris), IB (Fraccaro) and type II (Langer-Saldino, MIM 200610). A histological analysis of cartilaginous tissue is very helpful, especially to make the diagnosis of achondrogenesis type IB.[56] Achondrogenesis type IB is caused by the mutations of the diastrophic dysplasia (*DTDST*) gene.[57]

The *DTDST* gene encodes a sulfate transporter, which is required for the synthesis of sulfated proteoglycans in cartilage with other name, *SLC26A2*.[58] It is not surprising that defects of this gene result in a chondrodysplasia phenotype, although the precise mechanism whereby the impaired sulfation of proteoglycans causes abnormal enchondral ossification remains to be elucidated. Several mutations of the *DTDST* gene were reported in patients with achondrogenesis type IB, the most severe form, atelosteogenesis type II or neonatal osseous dysplasia (MIM 256050), an intermediate form and the mildest form of the diseases, diastrophic dysplasia (MIM 222600). The recessive multiple epiphyseal dysplasia (MIM 226900) is also caused by the mutations of *DTDST*. Homozygous mutant mice for the gene were characterized by growth

retardation, skeletal dysplasia and joint contractures, thereby recapitulating the essential aspects of the diastrophic dysplasia phenotype in man. The skeletal phenotype included reduced toluidine blue staining of cartilage, chondrocytes of irregular size, delay in the formation of the secondary ossification center and osteoporosis of long bones.

The cartilage hair hypoplasia (CHH, MIM 250250) or spondylometaphseal dysplasia McKusick type is characterized by disproportionate short stature, hypoplastic hair, ligamentous laxity, defective immunity, hypoplastic anemia, and neuronal dysplasia of the intestine (Hirschsprung disease). CHH was found to be caused by mutations in the ribonuclease mitochondrial RNA processing gene (*RMRP*).[59] The untranslated *RMRP* gene encodes the RNA component of a ribonucleoprotein endoribonuclease. Normally the RNase MRP complex is involved in multiple cellular and mitochondrial functions.

The difference between the Japanese and other ethnic groups is remarkable in terms of the mutations and polymorphisms in *RMRP* gene. Four novel mutations were reported in two patients with typical and atypical CHH.[60] A patient with typical CHH had a 17-bp duplication at +3 and a de novo 182G > A. The other patient with atypical CHH had a 17-bp insertion at -20 and a 218A > G.

The X-linked spondyloepiphyseal dysplasia tarda (SEDT; MIM 313400) has the features consisting of disproportionately short trunk, short stature, characteristic radiological findings of the spine (posterior hump, end plate sclerosis, and disc space narrowing) and the hips (short and thick femoral necks), and positive family history. The linkage analyses revealed that the *SEDL* gene on X chromosome is responsible for the disease.[61] The *SEDL* gene product, known as sedlin is a 140 amino-acid protein with a putative role in endoplasmic reticulum-to-Golgi transport. However, function of sedlin in bone and mineral metabolism remains to be characterized.

Leri-Weill dyschondrosteosis (LWD) (OMIM127300) is caused by the haploinsufficiency of the short stature homeobox-containing (*SHOX*) gene.[62, 63] LWD is an autosomal dominant form of mesomeric dysplasia first described by Leri and Weill in 1929. Patients with this condition demonstrate short stature due to shortening of the lower legs and Madelung deformity of the forearm. The abnormality of the *SHOX* also causes Turner skeletal features and a certain proportion of idiopathic short stature. The *SHOX* gene was cloned from the pseudoautosomal region of the sex chromosome (Xp22 and Yp11.3) and affects the adult height. It is exclusively expressed in the first and second pharyngeal arches and in the developing distal limb bones of the human embryo. The expression of SHOX is in the hypertrophic zone and less intensely in proliferating zone in growth plate. Although the function of SHOX in growth plate is to be elucidated, it may be involved in the fusion of growth plate and affect the growth at the pubertal stage.

Abnormality in PTH/PTHrP Receptor or G Protein

The family of G protein has many members, and Gsa is the representative protein which has been shown to cause bone diseases.[64] G protein is a signal effector molecule, typically forming heterotrimeric guanine nucleotide-binding proteins. Mutations in Gsa protein are divided into two groups, gain of function mutation and loss of function one. McCune-Albright syndrome and Albright osteodystrophy combined with pseudohypoparathyroidism are the diseases caused by these mutations, respectively.[65]

The PTH/PTHrP receptor is a G protein-coupled membrane-bound receptor and its best-characterized second messenger is cAMP. Although cAMP activates protein kinase A (PKA) as a downstream of PTHrP receptor, but it is still not clear what molecules responsible for delay of chondrocytes maturation serve as the substrates of PKA. In the growth plate, the PTH/PTHrP receptor mRNA is expressed in

prehypertrophic chondrocytes at higher levels. The critical role of the PTH/PTHrP receptor in endochondral bone development is highlighted by the discovery that two rare forms of chondrodysplasias, Blomstrand lethal chondrodysplasia (MIM 215045) and Jansen's metaphyseal chondrodysplasia (MIM 156400) are caused by mutations of this receptor. Blomstrand lethal chondrodysplasia is caused by inactivating mutations of the PTH/PTHrP receptor, while the mutated receptors found in Jansen's metaphyseal chondrodysplasia are constitutively active.[66, 67] The former disease is characterized by prenatal lethality, premature and abnormal bone mineralization and ossification, and shortened limbs. Endochondral bone formation is markedly advanced in fetuses with Blomstrand chondrodysplasia, and the columnar proliferative layer in the mutant growth plate is virtually absent. The disease appears to have an autosomal recessive pattern of inheritance. Jansen's metaphyseal chondrodysplasia is an autosomal dominant disorder characterized by short-limbed dwarfism secondary to severe abnormalities of the growth plate and hypercalcemia. The laboratory findings in patients with Jansen's metaphyseal chondrodysplasia are reminiscent of primary hyperparathyroidism. According to the original report, the genomic DNA from 10 JMC patients has been examined: seven patients had the H223R heterozygous nucleotide exchange, and three patients had distinct heterozygous mutations in the PTH/PTHrP receptor (T410P, T410R, and I458R).

When chondrocytes are forced to express either PTHrP or an activated form of its receptor, cartilage maturation is dramatically inhibited and bone formation delayed. Mice lacking either PTHrP or its receptor, in contrast, exhibit dwarfism of their long bones due to premature chondrocyte maturation. Therefore, PTHrP signaling negatively regulates the switch from a proliferative immature chondrocyte to a post-proliferative mature hypertrophic chondrocyte.

More recently, a heterozygous missense mutation Arg150Cys has been identified in the PTH/PTHrP receptor gene of two patients with enchondromatosis or Ollier and Maffucci diseases (MIM 166000). Enchondromas are common benign cartilage tumors of bone that can occur as solitary lesions or, in enchondromatosis, multiple lesions. This type of mutated receptor is constitutively active ***in vitro***. However, others reported that no mutations of the PTH/PTHrP receptor gene found in patients with enchondromatosis.

Pseudohypoparathyroidism, especially type Ia, is associated with the loss of function-type mutations in the Gsa gene (*GNAS1*).[65] In patients with the disease, no response to PTH is observed in the excretion of phosphate and cAMP. In contrast to McCune-Albright syndrome, the mutations are scattered along the entire gene, including missense mutations and deletions. This disease is inherited in a dominant fashion, and the mutation is found in a single allele. Albright hereditary osteodystrophy (AHO), which consists of short stature, shortening of the fourth and fifth metacarpals, round face, obesity and ectopic calcification is associated with pseudohypoparathyroidism type Ia (MIM 103580). Since the ***GNAS1*** gene is an imprinted gene, the transmission of the disease to the next generation is characteristic. Maternal transmission of Gsa mutations leads to AHO associated with resistance to parathyroid hormone (pseudohypoparathyroidism type Ia), while paternal transmission leads only to the AHO phenotype (pseudopseudohypoparathyroidism). Pseudohypoparathyroidism type Ib (MIM 603233) has the resistance to PTH only in kidney, and a region responsible for the disease is also associated with the ***GNAS1*** locus. PHP-Ib is caused by heterozygous mutations disrupting a long-range imprinting control element. Recently, deletions that remove the differentially methylated region encompassing exon NESP55 and exons 3 and 4 of the antisense transcript, those are located near ***GNAS1*** region. Thus, it is confirmed that epigenetic defects in the imprinted ***GNAS1*** cluster are associated with pseudohypoparathyroidism type Ib.[68]

McCune-Albright syndrome is characterized by three major symptoms, café au lait pigmentation,

multiple fibrous dysplasia and endocrine disorders including precocious puberty, hyperthyroidism, autonomous adrenal hyperplasia and growth hormone secreting pituitary adenoma. This syndrome is caused by the somatic mutation in the *GNAS1*gene, leading to elevated levels of cyclic AMP in cells. This gene has a hot spot of mutation at Arg of codon 201 (Arg201His or Arg201Cys).[69] Since these mutations are somatic, mosaicism for a mutation in the *GNAS1* gene is found in patients with McCune-Albright syndrome. Cells obtained from the lesions with fibrous dysplasia secreted a significant amount of interleukin (IL)-6 and exhibited an impaired response of IL-6 secretion to IL-1 in patients with McCune-Albright syndrome.[70] In response to the elevation of cAMP, IL-6 is produced perhaps through the cAMP response element of the IL-6 gene promoter. Fibrous dysplasia is a focal and benign fibrous bone lesions, which is caused by the activating mutation in the *GNAS1*gene. Hypophosphatemic rickets is sometimes observed as a complication of McCune-Albright syndrome. Two hypotheses have been put forward to explain the pathogenesis of hypophosphatemic rickets associated with McCune-Albright syndrome. One of them supposes the hypersensitivity to PTH in the renal tubules due to the mutation in the *GNAS1*gene, based on the inhibitory effect of PTH via cAMP on phosphate transport in the kidney. The other hypothesizes humoral factor(s) from the bone lesions which inhibit(s) phosphate reabsorption. In fact, Riminucci et al. recently reported the elevated plasma FGF-23 levels in patients with McCune-Albright syndrome.[71]

Hypophosphatasia

Hypophosphatasia (MIM 146300, 171760, 241500, 241510) is characterized by the hypomineralization of bone associated with the impaired activity of tissue-nonspecific alkaline phosphatase (TNSALP).[72] Hypophosphatasia has diverse phenotypes, and is usually classified into five subtypes based on the age of onset and clinical features; perinatal, infantile, childhood, adult type, and odontohypophosphatasia.[73] The severity of the disease is generally well correlated with the onset of the disease, except for odontohypophosphatasia, where only the teeth are affected. The patients with the perinatal type of hypophosphatasia almost always die around birth due to impaired development of the lung and the severe hypomineralization of their bones. However, the classification of these subgroups is not definite, and there is diversity in the phenotype which forms so-called spectrum. For example, we have previously reported a patient who achieved long-term survival without respiratory failure in spite of having fetal onset.[74] Since these patients survive longer despite their fetal onset, we should pay more attention to take care of them and to genetic counseling. The presence of such a case may depend on the development of a diagnosis procedure or on the specificity of the genotype. However, this benign form of hypophosphatasia found in the Japanese patients is surmised to be associated with a specific mutation, F310L, whose product retained approximately 70% of its enzymatic activity. The relatively high activity of the mutant enzyme may contribute to this relatively mild form. On the other hand, mutations found in the severe form of the disease do not tend to be associated with restricted positions, although the three-dimensional model study showed that most of the severe missense mutations were localized in crucial domains, such as the active site.[75] Elucidation of the molecular heterogeneity underlying hypophosphatasia may contribute to our understanding of the clinical heterogeneity observed in hypophosphatasia and the improvement of treatment, especially in patients with severe forms of hypophosphatasia.

The patients with the classical perinatal form of hypophosphatasia almost always die in utero or the neonatal period. Therefore, the perinatal form is a synonym for the lethal form. The second most severe form, the infantile type of the disease, is still associated with high mortality because of the impairment of respiratory function and hypercalcemia.[76] The patients with other types of hypophosphatasia usually do not suffer from life-threatening complications.

To date, more than a hundred mutations have been reported in the TNSALP gene in patients with hypophosphatasia, mainly in Caucasians and Japanese.[77] However, the mutations are scattered in the whole coding region, and only a few mutations have been recognized to occur frequently in the gene. Moreover, although the relationship between the clinical features and the mutation of the corresponding gene has also been analyzed, only a few reports detected the positive correlation between the mutations and severity.[75] The mutations responsible for mild hypophosphatasia may not cause a complete loss of ALP function, suggested by several reconstruction experiments in which mutated ALP activity was examined.

Although hypophosphatasia is usually inherited in an autosomal recessive manner, autosomal dominant inheritance is also recognized in some families with the mild form of the disease. Interestingly, the mutations in those families have been reported to show the dominant negative effect on the wild-type ALP activity, as one of the remarkable results of the recent progress in molecular biology.[78]

The mechanism for which the defect of ALP activity causes hypomineralization is not fully understood. There are three main assumptions for the role of ALP in mineralization process. First, ALP increases local phosphate concentration, leading to the acceleration of mineralization. Second, ALP decreases the concentration of local pyrophosphate, a substrate of ALP and an inhibitor of mineralization. Finally, ALP controls gene expression including osteopontin through the increase in intracellular phosphate concentration and affects the mineralization.

In patients with the infantile form of hypophosphatasia, bone marrow cell transplantation was tried to avoid the expected poor prognosis.[79] The patient was given T-cell-depleted, haplo-identical marrow from her healthy sister. Chimerism in peripheral blood and bone marrow became 100% donor. The patient survived and some clinical improvement was observed. More experience with transplantation of bone marrow or mesenchymal stem cells is necessary before the cell-based therapy becomes standard as treatment of severe hypophosphatasia.

Craniometaphyseal Dysplasia

Craniometaphyseal dysplasia (MIM 123000) is a genetic syndrome characterized by the sclerotic change of cranial and tubular bones that commonly present at a young age, often with facial abnormalities and otolaryngologic manifestations such as a conductive hearing loss. Craniometaphyseal dysplasia is defective in ANK, the pyrophosphate transporter.[80] Thus, this disease supports the inhibitory role of pyrophosphate in mineralization.

Diseases with High or Low Bone Mineral Density

Bone mineral density is determined by both genetic and environmental factors. The genetical approach has shown that multiple genes have effects on bone mineral density. Recent developments in molecular genetics and genomics have dramatically increased a power to identify genes which affect bone mineral density. The sclerosing bone dysplasias can be classified into two groups such as increased trabecular bone density (osteosclerosis) and a cortical bone thickening (hyperostosis). There are several diseases which belong to sclerosing bone dysplasias, e.g., osteopetrosis, pycnodysostosis and osteopoikilosis.

Osteopetrosis characterized by a marked increase in bone mass is a heterogeneous disorder of the skeleton in several animal species.[81] In humans, the disease is inherited as either an autosomal dominant or autosomal recessive trait. The autosomal dominant form exhibits mild symptoms only in adults (McKusick MIM 166600). The autosomal recessive form consists of three types; a mild type (MIM 259710), a lethal type (MIM 259720) which is rare, and infantile malignant osteopetrosis (MIM 259700) characterized by severe symptoms such as diffuse osteosclerosis of all bones, extramedullary hematopoiesis associated with bone marrow failure, and sensorineurogenic impairment in infancy. It is

caused by defects in bone resorption by osteoclasts, resulting in increased bone mass and narrowing of the bone marrow cavity. Impaired bone resorption, as seen in the various forms of autosomal recessive osteopetrosis (MIM 259700), can be due to mutations in a subunit of the vacuolar H^+ pump (*TCIRG1* gene) or to mutations in the chloride channel *CLCN7* gene in human.[82, 83] Another form of osteopetrosis is caused by carbonic anhydrase II deficiency, and associated with renal tubular acidosis and cerebral calcification (MIM 259730).[84] Very recently, mutation in the *GL* gene, the gene responsible for spontaneous mouse gray-lethal whose phenotype is a coat color defect and severe autosomal recessive form of osteopetrosis, leads to severe recessive osteopetrosis in human.[85] X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency, osteopetrosis and lymphoedema (OL-EDA-ID, MIM300301) is caused by hypomorphic mutations in NEMO, the regulatory subunit of the IKK (IkappaB kinase) complex encoded in the *IKBKG* gene.[86] Germline loss-of-function mutations in *IKBKG* are lethal in male fetuses.

Hematopoietic stem cell transplantation seems to be the most effective and rational therapy for malignant osteopetrosis at the moment, because it provides normal osteoclast precursors.[87] However, several kinds of treatment including high dose of active vitamin D and long-term therapy with interferon gamma (IFN-g), also have been reported to be effective.

Cathepsin K has been cloned as an osteoclast-specific cysteine protease. The abnormalities in this gene were reported in patients with pycnodysostosis (MIM 265800).[88] The characteristic features of this disease are disproportionate short stature, front-occipital prominence, high-arched palate, proptosis, a pointed nose and sclerotic bones.

In addition to impaired bone resorption, increased bone formation can result in sclerosing bone disorders. Recently, it is reported that the increased TGF-b signaling results in Camurati-Engelmann disease (MIM 131300).[89] The increased bone formation in sclerosteosis (SOST MIM 269500)[90] and in Van Buchem disease (MIM 239100) turned out to be due to loss-of-function mutations of the *SOST* gene, most likely increasing bone morphogenetic protein signaling. In addition, osteoprotegerin and receptor activator of NF kappa B (RANK), whose gene is TNFRSF11A, are responsible for juvenile Paget's disease and familial expansile osteolysis.[91, 92]

Infantile cortical hyperostosis (Caffey disease, MIM114000) is characterized by radiological evidence of cortical hyperostosis, soft tissue swellings, fever and irritability.[93] Caffey disease was first reported in 1945 by Caffey and Silverman. Its principle features include acute inflammatory manifestations and the early onset time, usually in the first year of life. The cause of the disease remains unclear and the disease is self-limiting.

Further, it was recently shown that the gene encoding the low-density lipoprotein receptor related protein 5 (LRP5) is one of the regulators of peak bone mass in vertebrates.[94] The autosomal recessive osteoporosis pseudoglioma syndrome (MIM 259770), a disorder causing both skeletal and eye abnormalities, is due to inactivating mutations in the *LRP5* gene.[95] Besides the neonatal blindness, children with OPPG have a very low bone mass and are very sensitive to fractures and skeletal deformities. The disease is not caused by abnormal collagen matrix, and this differentiates it from the severe osteogenesis imperfecta, although it shares similar phenotype with osteogenesis imperfecta. Of interest, obligate carriers of OPPG mutations show an increased incidence for osteoporotic fractures, indicating a dominant effect of this gene on bone mass. Targeted disruption of the *LRP5* gene in mice also produces osteoporosis postnatally. On the other hand, in a family that includes phenotypically normal individuals with exceptionally dense bones (high bone mass MIM 601884), a gain-of-function mutation (G171V) in the *LRP5* gene has been described.[96] The same mutation was found in another kindred with other phenotypic abnormalities, such as torus palatinus and a wide, deep mandible in addition to high bone density. LRP5 acts as a coreceptor for Wnt proteins and is expressed

in osteoblasts, where it is required for the osteoblast proliferation and functions in a Runx2-independent manner. These findings demonstrate that the LRP5 signaling pathway plays an important role in the regulation of bone mass in vertebrates.

LRP6 is another coreceptor of canonical Wnt pathway. Thus, it is interesting whether LRP6 is involved in bone metabolism, although a human disease caused by the abnormality of LRP6 has not been identified. Lrp6-null mice is embryonic lethal and not suitable for the analysis of bone metabolism. We found that a novel spontaneous mutation, ***ringelschwanz*** (***rs***) in the mouse is in the ***Lrp6*** gene.[97] While heterozygous rs animals appear normal, homozygous ***rs*** mutants show malformations in the vertebral column, digits and the neural tube as in ***Lrp6***-null mutants, these phenotypes are less severe as compared to those in null mutants. The ***rs*** mutation is regarded as a hypomorphic allele of ***Lrp6***. The Lrp6 dysfunction in ***rs*** leads to a delay in ossification at birth and to a low bone mass phenotype in adults. Thus, LRP6 is certainly involved in bone metabolism as well as LRP5.

Noonan syndrome

Noonan syndrome (MIM 163950) is an autosomal dominant disorder characterized by a variable phenotype comprising proportional short stature, congenital heart defects, and minor facial anomalies.[98] The main facial findings of Noonan syndrome are hypertelorism with down-slanting palpebral fissures, ptosis, and low-set posteriorly angulated ears with a thickened helix. Cardiovascular diseases including valvular pulmonary stenosis, atrial septal defect and hypertrophic cardiomyopathy (HCM) are observed in 50%~80% of patients with Noonan syndrome. Other symptoms are webbed neck, chest deformity, mild mental retardation, cryptorchidism, feeding difficulties, bleeding diathesis, and lymphatic dysplasias. The incidence of Noonan syndrome is estimated to be between 1:1,000 and 1:2,500 live births.

Recently, heterozygous missense mutations in the ***PTPN11*** gene, located on chromosome 12 (12q24), have been identified in 33%~60% of affected familial or sporadic cases of Noonan syndrome.[99-102] The product of the ***PTPN11*** gene, SHP-2, is widely expressed in various tissues and is involved in several signal transduction pathways in cooperation with growth factors, cytokines, and hormones. Thus, mutations in the ***PTPN11*** gene could cause the wide range of clinical features in Noonan syndrome. SHP-2 is composed of two tandemly arranged src homology 2 domains, N-SH2 and C-SH2, at the amino terminus, a single central phosphatase domain (PTP) and a carboxy-terminal tail (Fig. 63.3). In steady state, N-SH2 domain binds to PTP domain and suppresses its phosphatase activity. In contrast, the binding of both domains is loose and PTP exerts its activity in

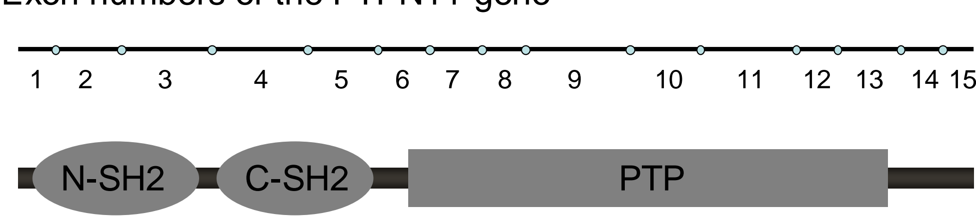


Fig. 63.3: The structure of the PTPN11 gene and functional domain structure of SHP-2 are illustrated in the Figure. The gene consists of 15 exons and the function domains of the SHP-2 protein comprise two tandemly arranged SH2 domains at the N terminus (N-SH2 and C-SH2) followed by a protein tyrosine phosphatase (PTP). The frequent mutations associated with Noonan syndrome are detected in Exons 3, 7 and 13 in the gene.

mutant SHP-2 found in Noonan syndrome. In other words, gain-of-function type mutation is found in the *PTPN11* gene in patients with Noonan syndrome. The structure of the *PTPN11* gene corresponding to the functional domain structure of SHP-2 is also depicted in the Fig. 63.3. The gene consists of 15 exons. The frequent mutations are detected in Exons 3, 7 and 13 in the gene.

To date, more than 70 mutations in the *PTPN11* gene have been described. Tartaglia et al. reported patients with *PTPN11* mutations were associated with pulmonary stenosis, while those without *PTPN11* mutations were with HCM.[103] Two other reports support the conclusion that HCM is found more frequently in patients with Noonan syndrome without *PTPN11* mutation. However, Sarkozy et al. showed LEOPARD syndrome (MIM 151100) with HCM was highly related to the mutations in exon 7 or exon12 of the *PTPN11* gene,[102] suggesting that HCM could be associated with *PTPN11* mutations.

As mentioned above, SHP-2 is widely expressed in various tissues and cell types, and has been implicated in diverse signaling pathways including those initiated by growth factors, cytokines and hormones. SHP-2 has compound signaling functions. For instance, SHP-2 directly interacts with growth factor and cytokine receptors when its ligand induces rapidly tyrosine phosphorylation. It can also interact with a variety of signaling intermediates such as Grb2, and Gab1 and 2. As a PTP, SHP-2 is believed to function by dephosphorylation of its associated signaling molecules, thus diminishing local signals. However, the ultimate effect of SHP-2 in most signaling pathways is to enhance the signal transduction, for example it activates the Ras-Raf-MAP kinase cascade. In most circumstances, SHP-2 plays a positive role in transducing signals relayed from tyrosine kinase-containing receptors.[103]

PERINATAL PERIOD

Skeletal Dysplasia, Lethal Type

The study in the St. John Hospital in Rome reported 14 cases of constitutional osteochondrodysplasias out of 2120 cases of newborns hospitalized (0.66%).[104] Among them, 4 patients (28.27%) belong to the group considered by the European Society of Pediatric Radiology as lethal. Representative lethal types of skeletal dysplasia and the corresponding causal genes are described in the Table 63.2. The genes responsible for osteogenesis imperfecta, thanatophoric dysplasia and hypophosphatasia are type I collagen gene, FGFR3 gene and tissue non-specific ALP gene, respectively, which are essential for the development of cartilage and bone, as described in the previous section. Other important molecules in enchondral bone formation such as SOX9 and PTHrP receptor are also responsible for lethal type chondrodysplasia. Patients with campomelic dysplasia caused by SOX9 mutations often have respiratory difficulties. Other lethal forms of short limb dwarfism consist of a lethal form of arthrogryposis multiplex congenita (MIM 108110), platyspondylic lethal osteochondrodysplasia, short rib syndrome and atelosteogenesis. Atelosteogenesis type 2 (AO2) (MIM 256050) is a neonatally lethal chondrodysplasia characterised by severe limb shortening and deficient ossification of parts of the skeleton. The disease is caused by mutations of the *DTDST* gene and its phenotypic overlap with non-lethal diastrophic dysplasia. The heterogeneous group of platyspondylic lethal skeletal dysplasias (PLSD) originally included thanatophoric dysplasias (TD1/2: MIM 187600, 187100) as the most common forms of this condition, as well as TD variants San Diego type (PLSD-SD: MIM 270230) and Torrance-Luton type (PLSD-TL: MIM 151210). The

Table 63.2: Skeletal dysplasia, lethal type and responsible genes

Disease	*Gene*
Osteogenesis imperfecta type II	COLI
Thanatophoric dysplasia	FGFR3
Hypophosphatasia lethal type	TNSALP
Achondrogenesis	COLII
Hypochondrogenesis	COLII
Achondrogenesis IB	DTDST
Camptomelic dysplasia	SOX9
Blomstrand chondrodysplasia	PTHR1
Short rib syndrome	?

FGFR3 gene mutations have been identified in TD1/2 and PLSD-SD, while PLSD-TL is caused by *COL2A1*. The metatropic dysplasia group includes fibrochondrogenesis, Schneckenbecken dysplasia and various forms metatropic dysplasia.[105] The perinatally lethal metatropic group of conditions consists of lethal metatropic dysplasia (Type 2) or hyperchondrogenesis, lethal hyperplastic metatropic dysplasia (Type 1) and fibrochondrogenesis. In our experience, osteogenesis imperfecta and thanatophoric dysplasia were the most frequent lethal skeletal diseases.

Amnionic Fluid

Clinical analysis of patients with skeletal dysplasias, lethal type revealed several characteristic phenotypes in each disease. For example, polyhydramnios is known as one of the features in patients with congenital anomaly. However, osteogenesis imperfecta and thanatophoric dysplasia were quite different in terms of association with polyhydraminios in our patients. In 11 patients with osteogenesis imperfecta, no polyhydramnios was observed. In contrast, nine out of 11 patients with thanatophoric dysplasia were complicated with polyhydramnios.

Intrauterine Growth Retardation (IUGR)

IUGR is often observed in fetus with severe skeletal dysplasia. Short limb associated with skeletal dysplasia tends to underestimate the expected body weight of the fetus. In contrast, hydrops, sometimes associated with achondrogenesis and thanatophoric dysplasia, affects the weight. Patients with osteogenesis imperfecta are often skinny and show IUGR.

Bone Age or Calcification of Bones in Fetus

In fetus, bone age can be estimated by the number of calcified bones whose calcification occurred during the gestational period. They consisted of talus, os calcis, ishium, os pubis, sternum, vertebral bodies (coccygeal and 5th sacral), lumbar transverse processes, proximal epiphysis of tibia and distal epiphysis of femur. We previously reported the relationship between the number of calcified bones and the gestational week. In hypophosphatasia, achondrogenesis and some forms of chondrodyplasia, calcification of bones is extremely delayed. In contrast, a mild decrease in number or normal development of calcified bones is observed in patients with osteogenesis imperfecta and thanatophoric dysplasia. These results suggest the difference in the pathogenesis of each skeletal dysplasia.

CLOSING COMMENTS

Due to the limitation of length of this review, the review cannot provide readers a whole picture accounting for the recent development of investigations on skeletal dysplasia. Apparently identification of genes responsible for skeletal dysplasia contributes to better understanding of the process in which chondrocytes, osteoblasts and osteoclasts proliferate, differentiate and interact with each other. Because the opportunity for pediatrician to take care of patients with skeletal dysplasia has increase, pediatrician should understand biological and developmental aspects of bone and chondrocytes as well as clinical advance of treatment of patients with skeletal dysplasia.

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