

The Physis: Fundamental Knowledge to a Fantastic Future Through Research

Proceedings of the AAOS/ORS Symposium

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Introduction

A proposal to the AAOS on behalf of the POSNA Education Committee (Chair Ken Noonan, MD), the AAOS, ORS, and NIH via an R13 grant mechanism, was supported to conduct a multidisciplinary symposium that discussed the current understanding of the growth plate, its pathology, state-of-the-art treatments, and future research directions. Never before on U.S. soil has an event like this transpired. The symposium was chaired by Drs. Matthew A. Halanski and Todd Milbrandt. The goals of the symposium were to:

1. Educate attendees on the current multidisciplinary knowledge of normal bone growth and development
2. Highlight various causes of abnormal growth and discuss available treatments and future possibilities
3. Identify key areas of focused research and establish multidisciplinary collaborations

In addition to formal didactic sessions, interactive discussions and networking opportunities were provided to allow cross pollination and collaborations between orthopaedic surgeons and other experts. More than 20 basic science and clinical faculty with expertise in biomedical engineering, cellular biology, developmental biology, endocrinology, genetics and gene therapy, molecular biology, nephrology, orthopaedic surgery, pediatrics, and stem cell technology, participated in the event.

Faculty included Michelle Caird, MD; Ernestina Schipani, MD, PhD; Henry Kronenberg, MD; Rosa Serra, PhD; Ola Nilsson, MD, PhD; Klane White, MD; Michael Bober, MD; Benjamin Alman, MD; Daniel Hoernschemeyer, MD; Francesco De Luca, MD; Jan-Maarten Wit, MD, PhD; Ken Noonan, MD; Neil Paloian, MD; David Deyle, MD; Shawn Gilbert, MD; Sanjeev Sabharwal, MD, PhD; Peter Stevens, MD; Jonathan Schoenecker, MD, PhD; Noelle Larson, MD; Todd Milbrandt, MD; and Wan-Ju Li, PhD

The purpose of this monograph is to summarize for the readers of *JPOSNA* that which was presented and discussed. It is an excellent primer for understanding growth plate function, pathologic states, and treatments.

The Normal Physis

Normal Growth Plate – Embryo to Adult

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Undifferentiated mesoderm from the somites (axial skeleton) or lateral plates (appendicular skeleton) condense and differentiate directly into either osteoblasts that form intramembraneous bone [in response to RUNX2 signaling], or into chondrocytes [through Sox 9 signaling]. Chondrocytes can further differentiate into osteoblasts though RUNX2. This two-staged process of bone formation, cartilage (chondrocytes) being replaced by bone (osteoblasts), is termed endochondral bone (“within the cartilage”) formation. The growth plate is involved in this type of bone formation.

Within the embryo, cartilaginous anlagen form from the mesoderm that are surrounded by a tissue called the perichondrium (“around the cartilage”). This initial anlage is avascular. Chondrocytes remain viable despite this avascularity due to Hif1, 2-alpha (hypoxic inducible factor). In the absence of these critical factors, widespread physeal chondrocyte death occurs resulting in short and deformed limbs. However, over expression of these factors results in delayed hypertrophy (Hif1) and ectopic chondrogenesis (Hif2). When blood vessel invasion occurs in the center of the anlage, oxygen is brought to the otherwise avascular cartilage. This new vascularity induces chondrocytes to differentiate into hypertrophic cells. The hypertrophic cells then either die and are replaced by bone or are transformed into osteoblasts and make bone (primary center of ossification). A separate blood vessel invasion occurs at the interface between the hypertrophic cells within the cartilaginous anlage and the perichondrium. This results in the formation of a bone collar as these peripheral hypertrophic cells lead to circumferential bone formation. Additional blood vessel invasion at either

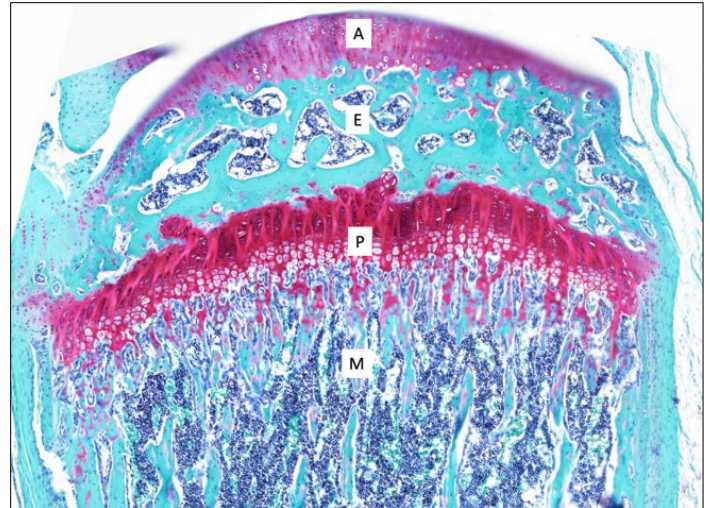


Figure 1. Histology of the immature murine proximal tibia. Notice the (A) articular cartilage; (E) epiphysis or secondary ossification center; (P) physis arranged in its typical columnar structure; and (M) metaphysis bone formed from the primary ossification center.

end of the cartilage anlage occurs, resulting in hypertrophic differentiation and bone formation (secondary center of ossification). The cartilage remaining between the primary and secondary centers of ossification is the growth plate or physis. Thus, the physis represents the remnant of the cartilaginous anlage that remains during childhood. The chondrocytes between the two centers of ossification arrange into zones based on their differentiation including the resting zone, proliferative zone, hypertrophic zone, and a zone of provisional calcification (Figure 1).

The physis itself is composed of cellular and structural/matrix components. Cellular components include resting, proliferating, and hypertrophic

chondrocytes located within their respective physal zones. In the zone of provisional calcification are dying hypertrophic cells and endothelial cells (capillaries, blood cells, osteoblasts, and osteoclasts); all of which take part in the complex process of replacing the cartilaginous template with bone. The acellular components of the growth plate are primarily hyaline cartilage consisting of 10-20% cartilage, 1-7% GAGS

(hyaluronic acid, aggrecan, chondroitin sulfate, keratin sulfate) and 65-80% water. The latter is drawn to the negative electric charges of the GAGS. Just as the cellular components differentiate and change through the different zones of the growth plate, the matrix also changes. For instance, Type II collagen is found in the proliferative zone, Type X in the hypertrophic zone, and Type I (bone) in the zone of provisional calcification.

Cells and Local Signaling

Henry Kronenberg, MD; *Massachusetts General Hospital, Boston MA*

Bone growth is a highly ordered process that develops out of the disordered mesenchymal condensations during fetal life. Between inception and birth, rapid proliferation and tremendous organization takes place to transform small mesenchymal collections of cells into bones. This complex process requires complex signaling to ensure that it occurs properly. During this period of time, three morphologies of chondrocytes exist, round proliferative (at the ends of the bones), flat proliferative, and hypertrophic (non-proliferating cells). Interestingly, the round proliferative cells proliferate radially to provide radial symmetry and later become the secondary center of ossification and the resting zone, whereas the flat proliferating cells proliferate and orient themselves in a columnar fashion that produces the longitudinal growth and shape of bones that we are used to. It is this columnar orientation that sets up the zones of the growth plate.

In addition to cell morphology, the differently shaped and positioned cells both produce and respond to factors produced differently. The round proliferative cells at the “top” of the growth plate produce PTHrP (Parathyroid Hormone related Peptide), which interacts on the PTHR (PTH-receptor) just as PTH does. Activation of the stimulatory G protein receptor on the proliferating

chondrocytes inhibits differentiation into hypertrophic chondrocytes. The result is continued proliferation until the flat chondrocytes proliferate “so far” away from the PTHrP-producing round chondrocytes that the PTHrP levels become low. This allows chondrocytes to differentiate further, resulting in chondrocyte hypertrophy through a Mef2c/RUNX2 pathway. If PTHrP levels are too low, or if the signaling pathway within the cell is damaged at any point (via genetic defects or pharmacology), chondrocyte proliferation is inhibited and premature terminal differentiation occurs, resulting in stunted growth. This can be seen clinically in multiple Brachydactyly Type E conditions.

As the flat proliferating chondrocytes grow out of the reach of the PTHrP-producing cells and hypertrophy, they start producing IHH (Indian Hedgehog). IHH signals back to the round proliferative cells to make more PTHrP; it also acts locally to encourage continued proliferation. Thus, it acts in a negative feedback loop with PTHrP. In addition, IHH produced in the hypertrophic zone interacts with the perichondrium through the RUNX2 pathway to differentiate cells into osteoblasts at the cartilage anlagen periphery to form a bone collar. Thus, IHH works together with PTHrP to ensure that the bone is growing (through cartilage

proliferation and hypertrophy) at the correct speed and the bone is being made at the right place. In addition to PTHrP, IHH, other factors [FGF 18 through the FGFR3

receptor, BMPs, Growth hormone/IGF1, and CNPs] interact to help control the growth process. The overall cellular regulation of the growth plate is highly ordered and highly complex.

Mechanobiology and Endocrine Regulation of the Growth Plate

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The ability of bone to react to mechanical load has been known for years. In 1892, Wolff described changes and increases in bone morphology and mass in response to load. Later, Hueter Volkmann described changes that occur at the growth plate under supraphysiologic load—growth at the physis is inhibited by compression and is stimulated with tension. Frost and Hamrick studied these effects further and noticed that the relationship is not linear at the growth plate.¹ Under physiologic conditions, tension (applied through muscles and tendons) and compression (gravity/weight) both increase growth rates. Yet a threshold of excessive forces can occur and then a precipitous decrease in growth occurs with further compression. Interestingly, growth inhibition also occurs without appropriate physiologic loading and can be seen in cases of fetal paralysis in which limbs are shortened and chondrocyte disorganization occurs^{2,3} and also in cases of hemiplegia related to cerebral palsy and polio.^{4,5}

Experimentally, physiologic load on the growth plate has been shown to be important as well. Paralysis models [temporary (botulinum toxin) or permanent (sciatic nerve transection)] in newborn mice can lead to decreased bone density (due to decreased weight bearing as a result of paralysis), decreased bone length but increased bone width.⁶ In these growth plates, researchers document a decreased number of proliferative cells, decreased hypertrophic zone (lower collagen X levels), and less columnar structure. Further

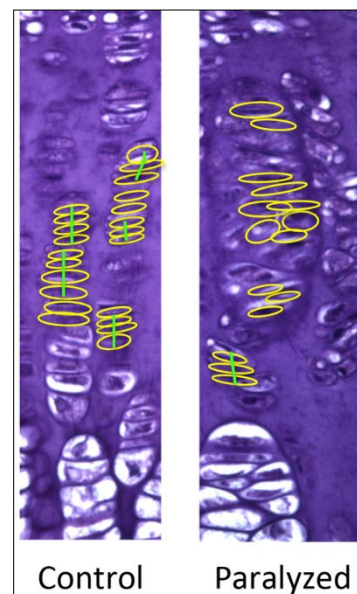


Figure 2. *Disorganization of Growth Plate Columnar Structure. The sciatic nerve for one limb of eight-day old mice was resected. Two weeks later, H&E staining of the sections of the growth plate indicated disorganization of the columnar structure. The control is the contralateral limb. Yellow circles are used to highlight individual chondrocytes.*

investigations into this disordered arrangement demonstrated that the normal longitudinal collagen fibers were lost and the normal columnar arrangement via cell division (vertical division followed by cell rotation) were affected and quantified by an altered morphometric index (Figure 2). Multiple disorders can change load, and also lead to physeal disarray.^{7,8}

From further work in this paralysis model, it appears that the actin cytoskeleton is altered with the unloading and that these changes may cause a disruption in the normal post-mitotic chondrocyte rotation leading to a disruption of the normal column formation as the actin cytoskeleton is important in cellular shape and response to

loads.⁶ The primary cilia is a structure found on nearly all cells and especially physal chondrocytes. This structure is thought to transmit chemical and mechanical signals from the surrounding environment into the cell. Deletion of the primary cilia was found to disrupt the normal actin cytoskeleton leading to similar

disorganization of the growth plate seen with unloading.⁹ In summary, the change in load (from the paralysis) may be detected and mechanotransduced through the primary cilia and actin cytoskeleton, altering the normal cellular rotation that occurs during cell division, resulting in physal disorganization (Figure 3).

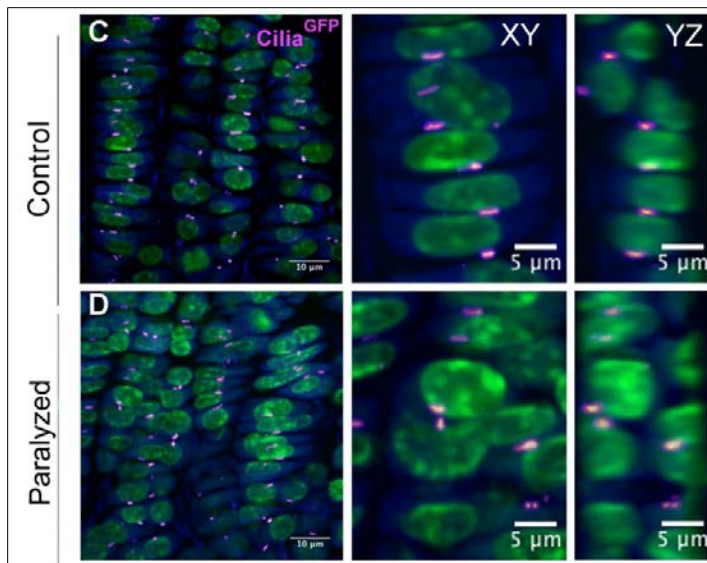


Figure 3. Primary Cilia on Growth Plate Chondrocytes. The sciatic nerve for one limb in eight-day old *Sstr3: GFP* mice was resected. All primary cilia in *Sstr3: GFP* mice are tagged with GFP. Sections from growth plate two-weeks after resection indicate orientation of primary cilia on chondrocytes. The images were pseudo colored so that primary cilia are pink, the nuclei are green and cytoplasm blue. Low magnification image is shown on the left. Higher magnification images in the XY and YZ planes are shown to the right.

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Growth from the physis boils down to three actions—cell proliferation, cell hypertrophy, and matrix production. Hormonal regulation of growth affects all three. When looking at all mammalian growth, a remarkable decline in growth rates occur from the fetal period, where growth rates of 100cm/year occur, to 50 cm/year at birth and continue a rapid decline, only briefly interrupted at puberty. Changes with age are functional (i.e., the rate of growth slows), as well as structural, as dramatic declines in cell numbers, growth plate height, and cell density occur with age. It appears the ultimate limit to growth appears due to quantitative and/or functional depletion of the growth plate progenitor cells of the resting zone.¹⁰

Through studies in rabbits, estrogen exposure causes a premature and permanent loss of resting zone cells, as well as a temporary reduction in growth rate (via decreased proliferation and hypertrophy) that is reversible.¹¹ Exposure to estrogen clinically appears required for ultimate growth plate senescence and closure. Failure to produce estrogen (through aromatase deficiency or estrogen resistance) results in continued growth well past puberty. Conversely, excessive estrogen very early in life via precocious puberty slows normal growth and results in earlier than normal growth plate closure, whereas exposure to estrogen later in development around the time of puberty results in final growth plate closure (Figure 4).

Other hormones have other effects on the growth plate. Excessive glucocorticoids or deficiencies in thyroid hormone result in slowing growth and delaying senescence. Under these conditions the growth plates appear “younger” than control growth plates of the same age. Interestingly, when the abnormality is corrected (either glucocorticoid removed or thyroid hormone restored), “catch up” growth occurs in which the growth rate is accelerated for a period of time until the ultimate predicted growth is achieved and ultimate senescence of the growth plate similar to that of the control growth plates.¹² Growth hormone and IGF-1 influence growth. Through the revised somatomedin pathway, it appears that bone GH, liver produced IGF-1, and local IGF-1 can all influence and increase growth at the growth plate. While proliferation may be increased, the major effects of IGF-1 appear to increase hypertrophy.

In summary, the growth plates have a limited growth capacity of which estrogen exposure appears to accelerate quantitative loss of resting cells and thereby accelerate growth plate senescence and cause ultimate physeal fusion. After transient growth inhibitions, growth plates are less senescent, and hence grow at a

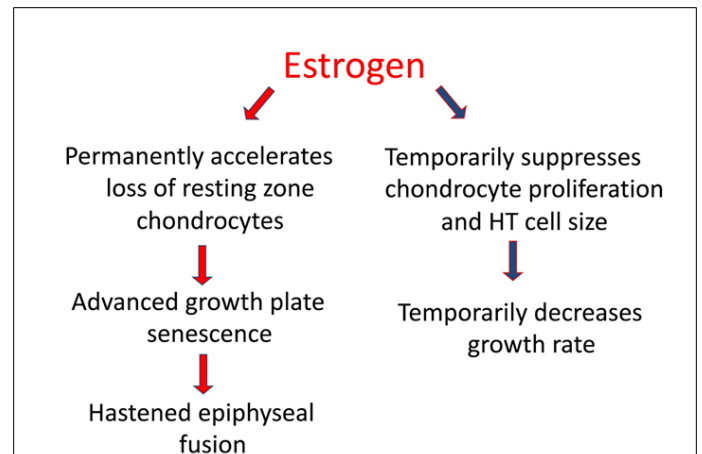


Figure 4. Model of estrogen effects on the growth plate. Transient exposure of estrogen in rabbits suggest that estrogen has reversible and permanent effects at the growth plate. Estrogen exposure temporarily suppresses growth rate through suppressed chondrocyte proliferation and decreased hypertrophic cell size. In contrast, estrogen permanently accelerates the loss of resting zone chondrocytes, accelerates growth plate senescence, and hastens epiphyseal fusion.

larger rate than expected for age, resulting in catch-up growth. If the growth inhibiting condition is severe and/or long-standing, the catch-up growth is often incomplete.

The Abnormal Physis

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As of 2015, there were 436 genetic skeletal disorders, of which 388 were associated with one or more of 316 genes. While much of the orthopaedic pathology may be similar in terms of genu varum/valgum, hip, and spine disorders, the genetic and physiologic differences are very important as different conditions may have markedly different severity of medical pathology as well as different inheritance patterns.

Abnormal Signaling – Prototype: FGFR3

FGFR3 (located at 4p16.3), is a transmembrane tyrosine kinase with three extracellular immunoglobulin domains. In normal conditions, binding of the receptor to its ligand causes receptor dimerization. This causes phosphorylation and pathway activation. In terms of growth, activation of the pathway inhibits growth. Thus, in the growth plate, the activation of the FGFR pathway

is the BRAKE of growth, whereas the CNP (C-natriuretic peptide) signaling pathway is the ACCELERATOR. Typically, this results in a thinner growth plate with smaller proliferative and hypertrophic zones. Disorders of the FGFR3 signaling tend to be a GAIN of FUNCTION mutation which leads to both ligand dependent and independent dimerization and downstream pathway activation. In addition to the growth plate, FGFR3 is found in multiple tissues including brain, lung, intestine, and kidney.

FGFR3 mutations lead to a wide spectrum of disorders which range from mild to severe. They are hypochondroplasia (mild), achondroplasia (#1 non-lethal skeletal dysplasia), SADDAN (Severe Achondroplasia, Developmental Delay and Anthracosis Nigricans), and the most severe, Thanatophoric dysplasia (#1 lethal skeletal dysplasia). These conditions are all inherited in an autosomal dominant pattern. Achondroplasia is 100% penetrant, and characterized by rhizomelic shortening with flatten acetabulae, ice cream scoop shaped proximal femurs, platyspondyly with short pedicles and a narrow intrapedicular distance. Thanatophoric dysplasia is characterized by a small bell-shaped chest with abnormal lung development, micromelic shortening, a cloverleaf skull with abnormal brain development and curved femurs (telephone handle femur) (Figure 5). In general, the differences in the genetic mutation are predictive of the severity of the phenotype. For example, 99% of children with achondroplasia have a G380R (glycine to arginine) mutation in which a (1138)Guanine (G) to Adenine (A) substitution occurs 98% of the time and a (1138)G to Cytosine (C) mutation 1% of the time. This mutation occurs in the transmembrane region resulting in approximately 80% baseline activation in the absence of the ligand, whereas the more severe Thanatophoric dysplasia results in even more abnormal signaling.

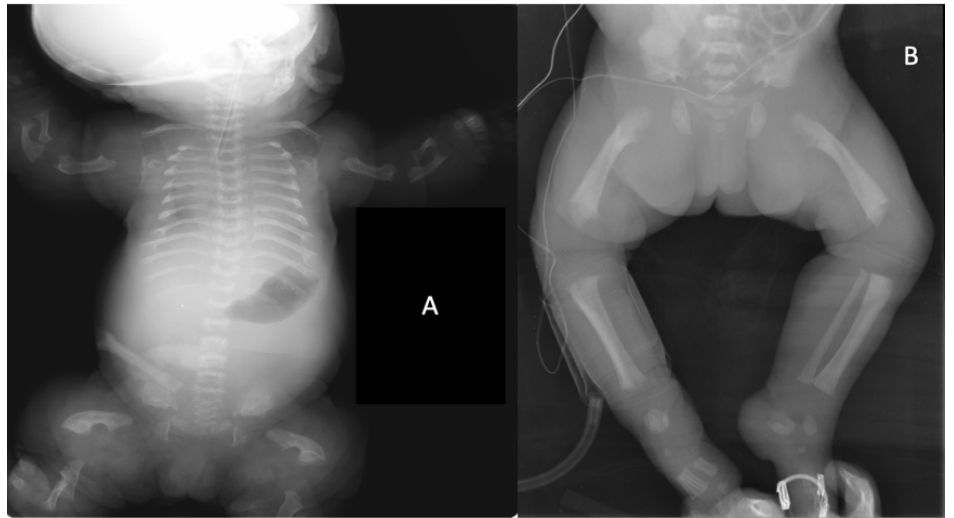


Figure 5. Radiographs demonstrating differences in FGFR3 mutations. (A) Thanatophoric dysplasia demonstrating the “telephone handle” femurs; (B) the “ice cream scoop” proximal femurs seen in the more common Achondroplasia.

Substrate Accumulation in the Physis - Prototype: Mucopolysaccharidosis (MPS)

Lysosomes are the cells recycling center with a pH that approaches 5.0. Many different materials are directed for recycling in the lysosome. When there is a defect in the recycling, a lysosomal storage disease occurs, as the cell cannot recycle the materials directed into the lysosome and these non-degraded materials accumulate in the lysosome and cell.

MPS are a group of disorders related to the recycling of (GAGs) of which Hexose and Hexosamine are recycled. Common MPS syndromes include Hurler (I), Hunter (II), Sanfilippo (III primarily neuro affected), and Morquio (IV A/B). Where the substrates accumulate determines where the pathology exists and can include the following locations: Respiratory, cardiac, corneal, hearing, dental, hepatosplenomegaly, MSK. Patients with Types I, II, and VI often have stiff fixed smaller joints like the hand (claw or fixed hand); whereas patients with Types IV and VII often exhibit hyperlaxity (possibility due to ligament detachment). Growth plates demonstrate accumulation of the materials.

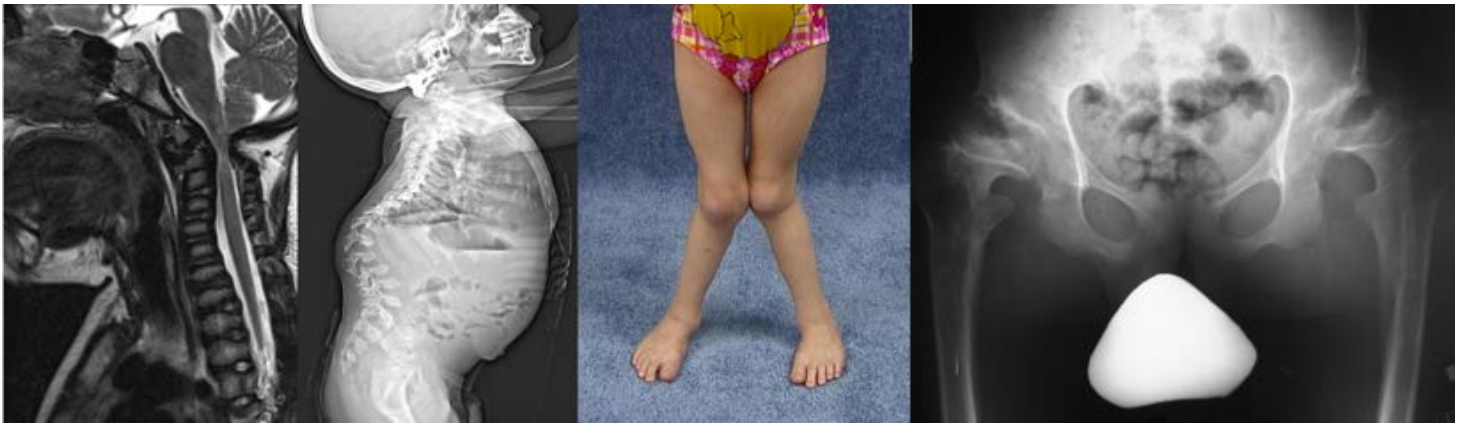


Figure 6. Orthopaedic findings in MPS IV. Myelomalacia caused by C 1-2 instability, stenosis, and extradural GAG deposition. Thoracolumbar kyphosis is also commonly observed. Lower extremity valgus is common with hips that are uncovered early in life and progress with additional lateral migration and eventual disappearance of the proximal femoral epiphysis (right).

Type IV is a short trunk dysplasia in which >90% have cervical spine instability, that when combined with a narrowing of the spinal canal due to GAG accumulation, can create a precarious situation and risk of spinal cord

injury. Therefore, cervical stabilization may be necessary. Thoracolumbar kyphosis is also common. Lower extremity deformities include extreme genu valgum and ankle valgus¹³ (Figure 6).

Genotype to Phenotype: Understanding Molecular Cause and Clinical Picture in Skeletal Dysplasia II

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There are hundreds of skeletal dysplasias and each effect the growth of the skeleton and physis in different ways. Skeletal dysplasias can be grouped and thought of in many ways: genotype, phenotype, structural, tumor related, developmental, protein processing, and by anatomic region of the bone. There are many genes that are expressed during normal limb and growth plate development. These protein products play crucial roles in cell differentiation and patterning. Understanding the proteins and how they function in the growth plate can help one understand when there is a genetic defect and the resultant skeletal dysplasia phenotype that presents.

Structural genes are genes that encode for proteins that provide the support or structure for the body. Oftentimes, these are autosomal dominant; phenotypes can be predicted by the protein involved and usually develop over time. One's lifespan can be decreased because the structure of the body can be severely affected and can cause compromise to the internal organs. Examples include Type II collagen mutations, which are important to resist compressive stresses in the body. There are more than 20 genetically confirmed conditions involving Type II collagen. These include achondrogenesis, Spondyloepiphyseal Dysplasia (SED),

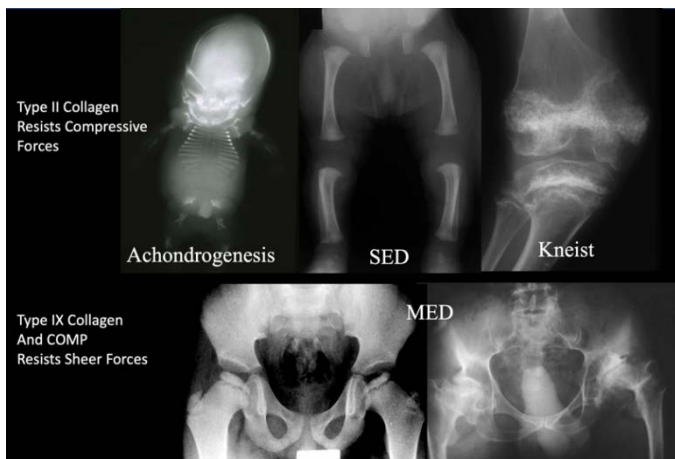


Figure 7. Radiographic findings in different structural related gene abnormalities. (Top) Type II collagen is important in cartilage and resists compressive forces, whereas (bottom) Type IX collagen and COMP proteins resist shear.

and Kneist dysplasia (Figure 7). Abnormalities in Type II therefore present with abnormal cartilage formation that is less resistant in compression. Defects in collagen Type IX or COMP mutations [such as seen in Multiple Epiphyseal Dysplasia (MED)] are also important as these normally act to support collagen Type II and resist shear forces. Thus, abnormalities in these proteins result in a less severe phenotype that demonstrate failure in shear forces. Often this results in progressive deformity over time.

Developmental conditions are due to a growth plate problem, due to patterning or the shape of the bone. The

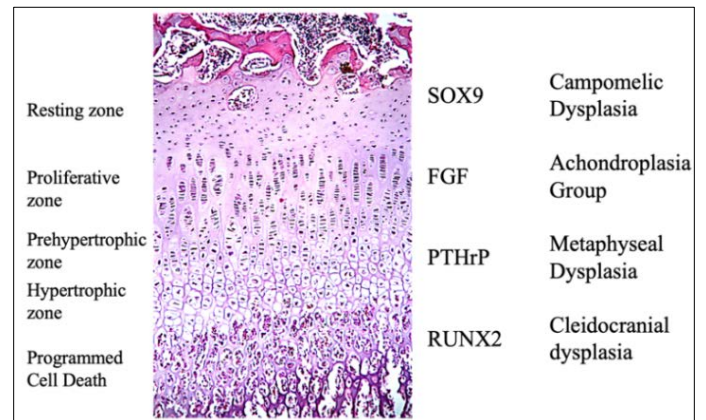


Figure 8. Demonstration of the different histologic regions of the growth plate (left), with important regulatory functions within the growth plate. Defects in these molecular developmental regulators result in different bone dysplasias (right).

phenotypes are present at birth and multiple organs can be involved. Different genes control the function within different areas of the growth plate. Mutations in different genes within different areas of the physis are associated with particular skeletal dysplasias (Figure 8). Metaphyseal dysplasias are usually due to altered terminal differentiation of the growth plate.

Enzyme deficiencies are often autosomal recessive and result in accumulation of products (MPS) inside of cells. This often results in avascular necrosis of bone or spinal problems. Enzyme deficiencies can be treated with enzyme replacement therapies.

Focal Overgrowth Problems

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Beckwith-Wiedemann syndrome (BWS) can be identified at birth because of neonatal blood sugar problems and large tongues. Children with BWS are predisposed to Wilm's tumors, present in up to 10% of patients, therefore, routine abdominal U.S. is

recommended. BWS is a problem with imprinting, and therefore the phenotype is different if it comes from the mother or the father. It involves IGF2; if you get it only from the mother then you end up with overgrowth. If you delete IGF2 from a mouse, then you get smaller

limbs with all zones of the growth plate being smaller. Proteus syndrome manifests with sole and palm skin changes along with overgrowth of the bones, fatty tumors and overgrowth of other soft tissues. The genetics is an activating mutation in AKT1. A drug that could block AKT1 could be a potential modulation in patients with Proteus syndrome.

Enchondromatosis can cause significant limb deformity and shortening resulting in complex deformities that

require complex reconstructions. The tumors have been found to have isocitrate dehydrogenase (IDH) mutations that the result in the ability of the enzyme to catalyze the NADPH-dependent reduction of alpha-ketoglutarate to D-2-hydroxyglutarate, which are active when the tumors become malignant.

There is one company that is working on IDH inhibitors; but so far there is no clinical data on its use in enchondromatosis.

Global Mis-Regulation: Short Stature vs. Gigantism

Francesco De Luca, MD; Drexel University, Philadelphia, PA

Longitudinal growth takes place at the growth plate (also known as the physis) through a two-step process called endochondral ossification. Cartilage formation (chondrogenesis) and replacement by bone tissue at the metaphyseal border of the physis (ossification) are tightly coupled. Thus, while the width of the physis remains relatively constant, the bone elongates.

The rate of endochondral ossification and, in turn, of longitudinal bone growth is regulated by multiple systemic (endocrine) and local (paracrine) factors. Endocrine factors (androgens, estrogens, Growth Hormone, Insulin-like Growth Factors or IGFs, thyroid hormones, and glucocorticoids) (Figure 9A) and paracrine factors (IGFs, Bone Morphogenetic Proteins or BMPs, C-type Natriuretic Peptide or CNP, Indian hedgehog or Ihh, Parathyroid Hormone-related peptide or PTHrP, and Fibroblast Growth factors or FGFs, among others) (Figure 9B) interact with one another and modulate the activity of several intracellular transcription factors (SOXs, SHOX, RUNXs, HDAC4, NF-

kB, etc.). The impaired activity of these regulatory networks leads to many disorders of stature, which can be classified into two broad categories: short stature, and gigantism (Figure 10).

Short stature can result from GH deficiency, IGF-1 deficiency (undernutrition, gene mutations of the GH signaling system), thyroid hormone deficiency (autoimmune, iodine deficiency, gene mutations affecting the thyroid hormone synthetic pathway) or

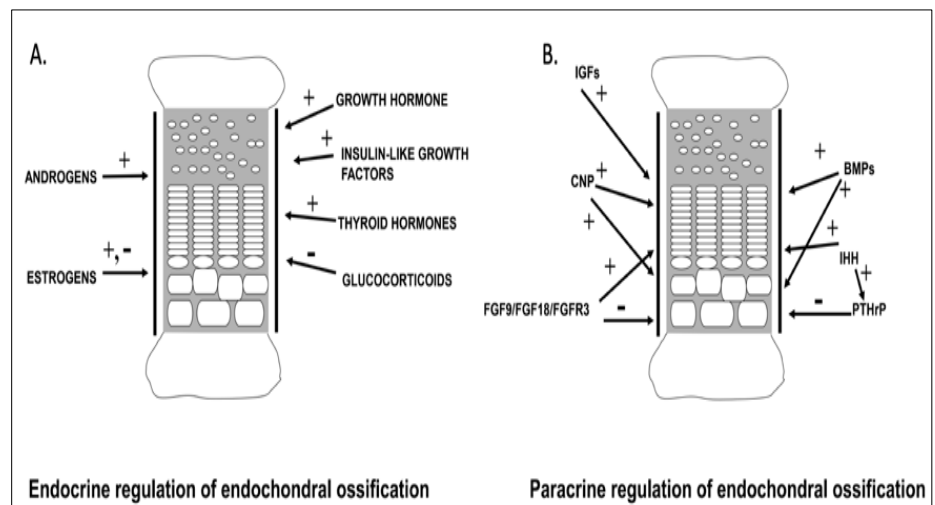


Figure 9. Schematics of the (A) Endocrine regulation of the growth plate and (B) the Paracrine regulation.

resistance (gene mutations of THRB or THRA), glucocorticoid excess (often iatrogenic, due to pituitary or adrenal adenoma, or secondary to gene mutations). Single gene mutations affecting the signaling systems of paracrine factors (CNP, PTHrP, FGFs) can cause skeletal dysplasia's associated with short stature, such as Jansen metaphyseal dysplasia, Achondroplasia/Hypochondroplasia, or Idiopathic Short Stature.

Gigantism is most often caused by GH excess due to single gene mutations or ectopic GH secretion from carcinoid or pancreatic neoplasms. Estrogen resistance or deficiency, and glucocorticoid deficiency, also result in extreme tall stature. Genetic disorders characterized by excessive activity of the CNP signaling system or reduced function of the FGF signaling system are also responsible for significant tall stature.

Effects of intracellular transcription factors on chondrocyte function in the physis

	CELL PROLIFERATION	CELL DIFFERENTIATION/ HYPERTROPHY	CARTILAGE MATRIX	APOPTOSIS
SOXs		+	+	
SHOX		?		+
RUNX2/RUNX3		+		
HDAC4		—		
MEF2C		+		
FoxA2/FoxA3		+		
HIFs		+		+
Ras/MAPK	+	—		
NFkB	+	+		—

Figure 10. The effect of different transcription factors on growth plate regulation.

The Sick Physis

Abnormal Bone Mineralization and Medical Treatment

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David R. Deyle, MD; Mayo Clinic, Rochester, MN

Bone is primarily comprised of Type I collagen and calcium and phosphate crystal hydroxyapatite. Collagen Type I is produced and secreted by the osteoblasts and is comprised of a triple helix (2 pro-alpha 1 (I) collagen chains and 1 pro-alpha 2 (I) collagen chain) that undergoes extensive extracellular modifications. These two chains are encoded by the COL1A1 and COL1A2 genes. Genetic abnormalities in these genes or other proteins involved in the production and assembly of the Type I collagen fibers results in Osteogenesis Imperfecta

(OI). The clinical severity of these abnormalities vary from mild (Type I) where only half the amount of pro-alpha 1 (I) collagen chain is produced to severe (Type 2 perinatal lethal) where there is disruption of normal triple helix folding leading to the production of abnormal Type I collagen. OI is characterized by bone fragility, low bone mass, increased bone turnover, and decreased cortical width and volume but other abnormalities such as dentinogenesis imperfecta, hearing loss, blue sclera, easy bruising, and scoliosis are also common.

The incidence of OI is roughly 1/20,000. In the past, diagnosis was made by skin biopsy; however, genetic testing has replaced skin biopsy. Using this technique, many newer subtypes of OI are being discovered. Currently bisphosphonates are used in children with OI to decrease bone pain and the number of fractures. These drugs act as an analogue to pyrophosphate and don't break down, therefore they inhibit osteoclast function. Clinical trials have demonstrated increased bone mineral density, the majority of which occurs in the first year of treatment. While widely used, concerns regarding long term use, bone healing and remodeling and avascular necrosis of the jaw exist. Other newer treatments include sclerostin antibodies that act to increase *wnt* signaling pathways, RANKL inhibitors to decrease osteoclast differentiation, and Teraparotide (PTH analogue) to increase bone remodeling, formation, and density. More recently stem cell therapies and CRISPR/Cas9 in vivo correction are being studied. In this work, genetically corrected cells would be injected into bones and would be incorporated into the bone as it remodels over time.

The mineralization of bone involves the normal deposition of hydroxyapatite from calcium and phosphate. The regulation of calcium and phosphate is rather complex and is regulated by a number of factors including vitamin D. Vitamin D can be ingested in either the D2 or D3 form or produced in the skin when exposed to UV light as D3. These biologically inactive forms must undergo two hydroxylation reactions. The first hydroxylated mediated by the 25-hydroxylase occurs in the liver to generate 25-hydroxyvitamin D, and the second mediated by 1 α -hydroxylase occurs in the kidney to create 1, 25-dihydroxyvitamin D. The 1, 25-dihydroxyvitamin D form of vitamin D is the active form in calcium regulation. It acts to increase calcium absorption from the gut and prevent calcium excretion in the kidney. PTH or parathyroid hormone also is involved in maintaining calcium levels. It is secreted when serum levels of calcium are low. It increases

calcium levels by (1) increasing the activity of 1 α -hydroxylase in the kidney, therefore indirectly increasing absorption through increasing active vitamin D levels, (2) acting on the kidney to keep calcium in the serum and dump phosphorous in the urine, and (3) activating osteoclasts to resorb bone to free up calcium into the serum. Thus, from an orthopaedic standpoint, it mobilizes calcium from the bone and inhibits hydroxyapatite formation by eliminating phosphorous. Finally, FGF-23 (fibroblast growth factor) is made by osteocytes in response to activate vitamin D and high phosphorous levels. Fgf-23 acts on the kidney to increase the excretion of phosphate.

Abnormal mineralization can occur from 1) insufficient levels of either mineral or 2) an inability to maintain the hydroxyapatite locally within the bone. Low calcium (Calcipenic Rickets) occurs from low vitamin D or calcium intake or genetic abnormalities in its production or receptors (vitamin D resistant). The effects of decreased mineralization on growing bones is a thickened physis and metaphysis with unossified osteoid that is mechanically weaker than normal bone. This results in widening of the physis and metaphysis, bowing of the weight bearing bones, the "rachitic rosary" of the ribs, and frontal bossing of the skull. Vitamin D is low in most cases and PTH high; serum calcium can be low to normal as PTH is driving the mobilization of calcium from the bones. Treatment is nutritionally based with replacement of vitamin D and calcium. In cases of genetic defects, it is important to know what form of vitamin D must be given to counteract the defect. In cases of vitamin D resistance, elemental calcium is often required.

Low phosphate can also result in similar clinical findings. Hypophosphatemic rickets occurs most commonly due to a genetic defect in the PHEX gene and is known as X-linked hypophosphatemic rickets. PHEX regulates the FGF23 levels and defective PHEX results in excessive FGF23. This results in massive phosphate dumping in the urine by the kidney. Similar phosphate

wasting is also seen in Fanconi's anemia where proximal renal tubular issues result in phosphate dumping. Regardless, the diameter of the growth plate increases, and bowing is observed. High FGF23 and low phosphate are found in the blood. Diagnosis is usually based on laboratory findings and family history and confirmed by genetic testing. Recently a monoclonal antibody (burosumab) has been developed against FGF23 to block its effects and early results appear promising.

Renal osteodystrophy (or Chronic Kidney Disease-Mineral Bone Disease) is a complex disorder that can result in the demineralization of bone and increased ectopic ossification of arteries. Low serum calcium and high levels of phosphate can occur when the proximal tubules that normally produce the 1 α -hydroxylase are injured and cannot excrete phosphate. These high levels of phosphate and low levels of calcium increase serum PTH resulting in a secondary hyperparathyroidism (osteitis fibrosis cystica). This complicated interaction can lead to dynamic bone disease, osteomalacia, rickets, and mixed osteodystrophy. Often the focus of treatment is to give binders (in the past aluminum) to decrease the elevated phosphate levels.

Local abnormalities can affect the mineralization of bone as well. Pyrophosphate inhibits hydroxyapatite

production and thus ossification. When there is a defect in the alkaline phosphatase enzyme, pyrophosphate levels increase thus inhibiting mineralization in two ways 1) by directly inhibiting the production of hydroxyapatite and 2) by decreasing the available phosphate substrate. The diagnosis can be difficult as many labs lack a lower limit for alkaline phosphatase levels, thus the ultimate diagnosis is made by genetic testing. Radiographically focal defects resembling a "tongue" can be found in the metaphysis. Clinical severity can vary as a perinatal (lethal), infantile (high calcium, kidney disease, rickets, rib fractures), childhood (early loss of baby teeth with roots intact, joint pain, rickets), and adult form (osteomalacia and pseudogout). Recently an enzyme replacement therapy that targets and delivers the enzyme (alkaline phosphatase) to bone has been developed (Asfotase) which can decrease the pyrophosphate and PLP pyridoxal 5'-phosphatase levels and improve bone mineralization.

Glucocorticoids can lead to decreased mineralization as well. Soon after the initiation of steroid treatment, the steroids inhibit the 1 α -hydroxylase which results in decreased active vitamin D and a rapid calcium wasting in the urine and increase in PTH, which occurs rather rapidly. Long term, the steroids act to inhibit osteoblasts and activate osteoclasts.

Obesity and the Physis: Pathophysiology and Clinical Effects I & II

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Obesity rates around the world and especially in the U.S. are increasing. Associated with this is the metabolic effects of hypertension, pro-inflammatory state, hyperlipidemia, insulin resistance, and diabetes. Thus, as it pertains to the growth plate, there is the mechanical

effects of increased load on the bone and the endocrine effects on the physal chondrocytes. Leptin is a protein produced by adipocytes. When functioning properly, increased Leptin levels act to suppress hunger centrally. In the physis, Leptin appears to promote

chondrocyte proliferation and increases physal thickness at low doses but has little effect at high doses. Clinically higher Leptin levels have been found in both obese and non-obese children with SCFE. Diet induced obesity led to insulin resistance in mice. At the growth plate, the high insulin level led to accelerated growth and increased growth plate height that can be mitigated with insulin sensitizers. Insulin receptors have been found to exist on physal chondrocytes. A correlation of hypertension, which is often seen in obese children, may also affect the growth plate. Whether it is the effects of the mechanical load, altered insulin levels, or increased estrogen levels due to the adipocytes, precocious puberty is often seen in these obese patients (up to 24 months advanced in early onset Blount's) leading to premature physal closure.

From a clinical pediatric orthopaedic standpoint, obese patients have an increased risk of fracture at the physis with odds ratios of distal femoral physal fractures to be six times that of non-obese children and tibial fractures five times more than non-obese children. Attempts in rodent models to determine how dietary obesity structurally effects the physis and the mechanical properties of the bone have been challenging; however, it appears that growth acceleration and increasing physal thickness, most likely occur early in this process.

Outside of fractures, clinical pathology about the growth plate is seen in children with obesity including early and late onset Blount's disease and Slipped Capital Femoral Epiphysis (SCFE). While histologically there may be similarities in loss of normal architecture and columnar formation, differences between these entities exist. In SCFE, increasing BMI increases the likelihood of a slip at a younger age and also the likelihood of bilateral involvement. However, in Blount's disease this is not the case. Increasing BMI does not appear to increase the likelihood of bilateral involvement. Despite what families and surgeons may think, correction of the

deformities does not predictably lead to improvements in BMI.

The effects of obesity and iatrogenic loads from limb lengthening allow insight into the effects of mechanical load at the growth plate. From the Frost paradigm stating that physiologic amounts of compression and distraction promotes growth, one can understand the Selenius curve of the physiologic maturation of lower limb alignment. As the child begins to walk, and the legs are in a varus position, the physiologic loading medially, may promote medial growth to slowly correct the small amount of varus present. Similarly, when the child is older the same occurrence may occur laterally to correct the valgus typically encountered at age three, until arriving at the "normal" alignment by age seven. However, if the load is greater than physiologically normal, growth is inhibited and the resultant deformity sets up a vicious cycle of more load and more inhibition, resulting in the progressive deformities clinically encountered. If the BMI is greater than 40-45, trying to rescue the physis and correct the alignment with guided growth techniques becomes very difficult.

Observations of growth and the physis following limb lengthening allows one to study the effects of the loading on the growth plates. Interestingly, just as differences are seen in the femur (SCFE) and the tibia (Blount's) with regards to pathologic overloading due to obesity, the bones respond differently to lengthening. Lengthening the femur appears to have no effect or a growth promoting effect on subsequent growth. Whereas the tibia results in diminished growth if lengthened twice or in conjunction with the femur. In rabbit models, this growth inhibition following lengthening was observed and could be histologically alleviated by soft tissue release suggesting that decreasing the soft tissue tension across the physis decreases compression across the physis. Why this appears isolated to the tibia is unknown. Stokes et al.

looked at the direct effects of loading and distraction of the physis of both the spine (tail) and tibia and found that compression affected the growth plates function by diminishing the growth rates and that this effect was

more pronounced in the tibia than the tail. Furthermore, static compression had a greater effect than cycled (diurnal compression), increasing compression from 0-10-30N led to decreased levels of Type II and X collagen production.

The Injured Physis

Physeal Injury and Effects on Growth

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Up to one-third of all children will suffer a fracture and of these, 10-30% will involve the physis, with the fingers followed by the distal radius being the most common locations. Of all physeal fractures, 4% will be associated with a physeal arrest. The Salter Harris classification system is the most commonly used system describing fractures about the physis. Type I is a fracture through the zone of hypertrophy, Type II involves the metaphysis, Type III involves the articular surface, and Type IV fractures occur through all the zones (including resting and proliferative zones) and include the metaphysis and articular surface. A Type V involves a crush injury to the physis. Development of a bar depends on multiple factors including patient age, injury mechanism, fracture pattern, anatomic location, and treatment. (Figure 11). On a histologic level, fractures not involving the proliferative and reserve zones tend to do well. Similarly, despite contradictions in the literature, recent clinical and basic science studies would indicate the interposition of periosteum alone is not a risk factor for bar development, but rather the energy of injury. While

injuries to the growth plates of phalanges and radius appear resistant to bar formation, bars are common in the distal tibia with up to 25% of all distal tibial fractures resulting in a bar and 30-60% of all Type IV medial malleolar fractures (“trampoline injuries”).

Other fractures adjacent but not directly affecting the growth plate have demonstrated the ability to actually accelerate growth leading to limb length deformities or angular deformities, the most notable being the “Cozen’s” fracture of the proximal tibia (Figure 12). Often these growth disturbances are temporary and respond to conservative treatment.



Figure 12. An example of Cozen’s fracture. Minimally displaced proximal tibia fracture healed without issue; however, medial sided overgrowth caused a pronounced valgus deformity that spontaneously corrects over time.

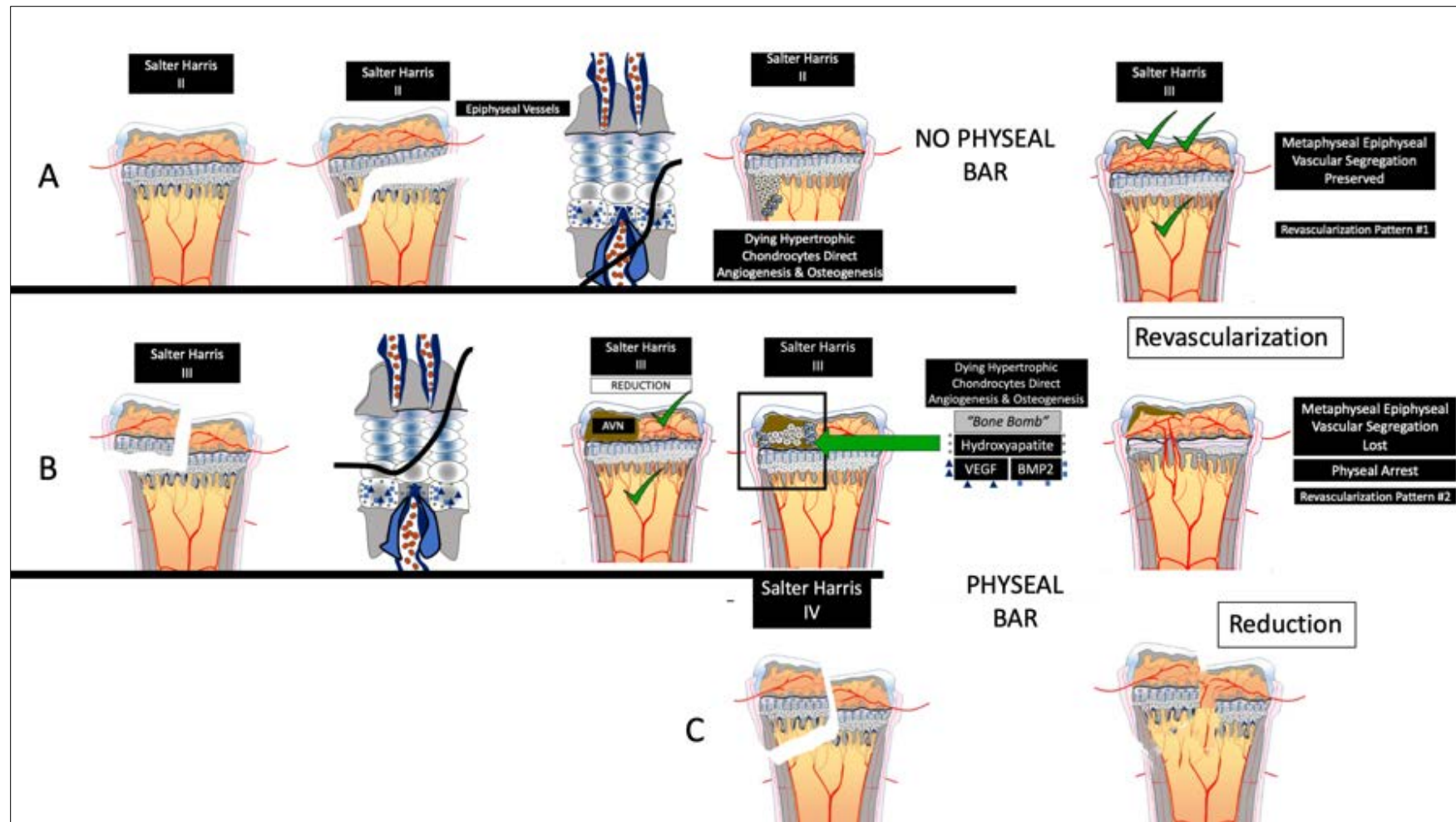


Figure 11. Schematic of Physeal Fractures and Bar formation. (A) Salter Harris I and II fractures traverse the hypertrophic zone and metaphyseal bone. Epiphyseal and metaphyseal blood supplies stay segregated and the physeal chondrocytes are present to heal the fracture. In type III and IV fractures (B, C), the fracture line traverses the proliferative and resting zones and through the secondary ossification center (epiphysis). Depending on the energy and location of the injury, vascular segregation may be maintained during revascularization and healing may be uneventful (B top) without a bar forming. However, if the epiphysis revascularizes through the metaphysis, a bar formation may occur. Anatomic reduction may decrease the fracture gap and help prevent metaphyseal patterned revascularization (Figure adapted from J. Schoenecker).

Infection, Neoplasm, and the Effects of Treatment on the Growth Plate

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Although metaphyseal and epiphyseal infections and neoplasms can directly invade the growth plate, more commonly these pathologies disrupt the vascular supply of the physis, potentially leading to a physeal bar. Many of the growth disturbances seen in both of these conditions (and likely others) is likely secondary to the small vessel disease that occurs as a result of having an increased thrombotic state (as a result of a prolonged period in the acute phase reactant response of both of these pathologies) that leads to altered blood supply to primarily, the epiphysis. The degree to which the epiphyseal blood supply is lost, and perhaps more importantly, the origin of the restorative blood supply that returns are important in determining the sequelae on the physis and growth.

The physis is essentially an avascular, hypoxia cartilaginous disk, existing between two, segregated, highly vascular regions of bone including the metaphysis and the epiphysis. In these highly vascular bony areas exists endothelial cells (which bring the oxygen in the blood) and the osteoblasts which require high oxygen

and cannot exist more than 200 microns away from the endothelial cells. The chondrocytes within the physis rely on the diffusion of oxygen from the epiphysis and thrive in this hypoxic environment. As they proliferate away from their blood supply they hypertrophy. These hypertrophic chondrocytes direct ossification and are “Bone Bombs” as they contain everything necessary to make bone as they are angiogenic (VEGF) and osteogenic (BMP’s and hydroxyapatite). It is thought that the touch of a metaphyseal endothelial cell (or the increase in oxygen that occurs with endothelial cells during growth) is what lights the fuse on the bone bomb to release its contents (Figure 13). Thus, the hypertrophic chondrocyte differentiates in the most hypoxic portion of the physis and directs the vascular ingrowth and ossification from the metaphysis. The very tightly controlled proliferation and hypertrophy ensure the proper rate of growth and separation to keep the two vascular supplies segregated. At the time of growth plate closure, the rate of growth diminishes and the segregation is lost.

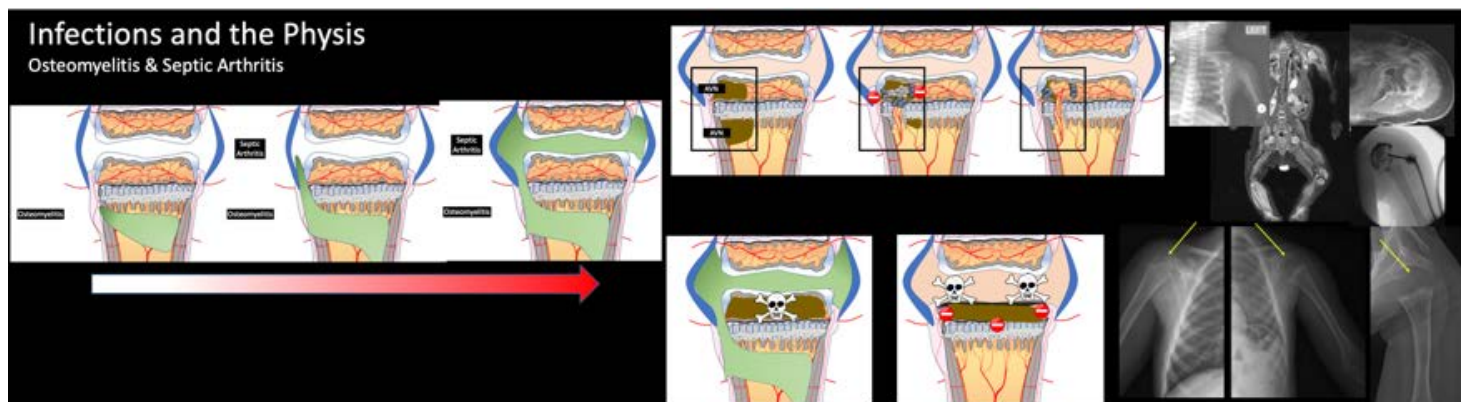


Figure 13. Metaphyseal Osteomyelitis may expand into the adjacent joint causing septic arthritis. The infectious process may lead to focal areas of AVN in the metaphysis and physis (top row) or global AVN of the epiphysis (bottom row). Case example (far right) of septic arthritis of the shoulder in a 26-day old, resulting in global involvement of the left proximal humeral epiphysis (arrows) despite I&D (clinical case provided by N. Larson).

In cases of osteomyelitis and septic arthritis (or in neoplasms or its treatments), microvascular disruptions occur in either the metaphysis, the epiphysis, or both. If these occur in the metaphysis, bone (and the oxygen loving osteoblasts) may be lost and resorbed. In this state the area is “filled in” by hypertrophic chondrocytes. Upon reaching the area of the metaphysis that still has a blood supply, the “bone bomb” is set off and the defect is filled. When this occurs in the epiphysis however, any area of the growth plate that would receive its oxygen through diffusion loses its source. In that region the oxygen diffusion gradient within the growth plate is lost

and either causing a loss of, or more likely the differentiation of resting and proliferative chondrocytes into hypertrophic chondrocytes. This results in the “bone bombs” being placed throughout the physis, and recruitment of endothelial cells into the physis. As a result, the loss in the vascular separation of the epiphysis and metaphysis will ultimately lead to the formation of a bony bar crossing the physis as the metaphyseal blood supply restores the blood supply to the epiphysis (Figure 13). If complete epiphyseal blood supply is lost and metaphyseal blood cannot penetrate the physis to restore it, avascular necrosis occurs and complete loss of the epiphysis and joint surface may occur.

Therapeutics/Treatment for Physeal Disorders

Current and Future Approaches to Improve the Physis in Skeletal Dysplasia

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Skeletal dysplasias are genetically determined diseases that ultimately result in abnormal skeletal architecture and physeal dysregulation, with the resultant short stature conditions that occur.¹⁴ The growth plate has several therapeutic challenges as the physis is relatively avascular, the disease state occurs early (prior to birth or shortly after birth), and the sequela of the bone are not addressed by medications and will still potentially require surgery to correct the deformity. Medical therapies that have been tried include small molecule therapies (such as biochemical regulators), anti-inflammatory therapies to treat downstream effects, antibody therapies, and gene-mediated therapies. Inflammatory disease is a common pathologic endpoint of many genetic mutations affecting the growth plate. Consequently, already approved medications such

as TNF- α inhibitors, may play a therapeutic role for many of these disorders.

Achondroplasia is the most common form of skeletal dysplasia and results from point mutations in the FGFR3 receptor protein and an inhibitory upregulation.¹⁵ Therapies are currently being developed that can be FGFR3 specific or can target the FGFR3, tyrosine kinase signaling pathway further downstream. C-Natriuretic Peptide (CNP) inhibits the MAPK pathway at RAF1.¹⁶ Mouse models demonstrate an improvement in the length of long bones and in the organization of the physis histologically.¹⁷ Clinical trials are now underway for treatments of achondroplasia. Early human trials are encouraging in terms of safety and efficacy.

The mucopolysaccharidoses are a family of disorders in which a single gene (enzyme) defect results in failure of glycosaminoglycan (GAG) metabolism. Secondary biochemical effects of GAG accumulation inhibit SOX9 function. Chondrocytes fail to transition from the proliferative phase to the hypertrophic phase in the physis with failure of ossification of physeal cartilage¹⁸. TNF- α inhibitors have shown promise in animal models

and small case reports.¹⁹ Gene therapy has been tried in a dog model which has shown a profound functional benefit, but no clear benefit to bone disease thus far.²⁰ X-Linked hypophosphatemic rickets does not directly affect the physis but there are physeal implications in growing children.²¹ Monoclonal antibodies targeting FGF23 have demonstrated significant radiologic benefits to physeal morphology and growth, now with FDA approved therapy.

Medical Treatments for Global Physeal Misregulation

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Growth hormone (GH) injections have been used since 1958 for children with GH deficiency as replacement therapy which was pituitary derived GH until 1985. Since 1985, recombinant human GH has been used. GH is also used as enhancement therapy for multiple growth disorders: chronic renal failure, Turner syndrome, Prader-Willi syndrome, idiopathic short stature, SHOX haploinsufficiency and Noonan syndrome. In severe GH deficiency, GH leads to a fast-catch-up growth followed by normal growth and a normal adult height. In non-GH deficient conditions growth velocity increases in the first few years of treatment and the effect on adult height is approximately 1 SD.

Estrogen has a non-linear effect on growth and skeletal maturation. In patients with Turner syndrome, very low doses of estrogen combined with GH have a small positive effect on growth and adult height gain. In patients with hypogonadism, there is a neutral effect on growth and maturation. High dose estrogen treatment in girls with extremely tall stature results in inhibition of growth and acceleration of skeletal maturation which

results in reduction of adult height; this is no longer routinely used as it can have a negative effect on fertility in these girls.

Androgens are used for hypogonadism as a substitution dose or can be used as for patients with constitutional delay. In both conditions, androgens have a neutral effect on growth and skeletal maturation. Higher doses can be used for limiting adult height in extremely tall male adolescents and can result in a reduction in adult height. There are significant side effects and therefore this is not used frequently. Anabolic steroids have been used in constitutional delay and have little effect on growth velocity and have no effect on adult height. The anabolic steroid oxandrolone has been found to increase height by 3cm in Turner syndrome patients in addition to GH.

Gonadotrophin-releasing hormone agonists (GnRHa) are established for use in patients with precocious puberty and can be utilized in addition to GH in adolescents with a very low predicted adult height by delaying bone maturation, resulting in increased adult height.

Current and Future Treatments of the Injured Physis

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What is a “bony bar” or a growth arrest? In terms of histology, a bar is bone that forms between the metaphysis and epiphysis. Animal studies have demonstrated that this is woven bone that forms through intramembranous ossification whose trabeculae are parallel to the mechanical axis. Clinically bars are detected from angular deformities or differences in limb length discrepancies using a variety of radiographic techniques. Often the Park-Harris growth arrest (or resumption) lines can be seen as a linear radiodensity in the metaphysis following a fracture. When these lines are parallel to the adjacent physis, the clinician is reassured symmetric growth is occurring; however, when these lines point at or intersect the physis suspicion of an arrest should be high.^{22,23}

When a bar is suspected, either CT (gold standard) or MRI mapping of the physis and the bar can be completed so that the cross-sectional area of involvement, presented as a percentage of involvement (area of bar/area of physis X 100), can be determined. In general, patients with >2 years of growth and <50% of physal involvement can be considered for physal bar excision. Patients with >50% involvement or <2-years of remaining growth are better candidates for other procedures such as ipsilateral and contralateral epiphysiodesis, osteotomies, and/or distraction osteogenesis. In addition to

providing the area involved, the advanced imaging can be useful in describing the location of the bar (peripheral, linear, central), and can greatly aid surgical planning if bar resection is pursued.

Bar resection has been described by H. Peterson (Figure 14), and involves making a large metaphyseal cortical window, through which a path is made down to the physis. The bar is then resected under direct vision using a high-speed burr, until normal physis is visualized circumferentially.²³ While different interposition materials have been tried, Cranioplast, in our experience has had the best and longest track record. While satisfactory results can be had using this technique, the time between the injury and the bar resection appears important. However, despite restoring growth, often this growth is not normal and additional surgical procedures may be needed.

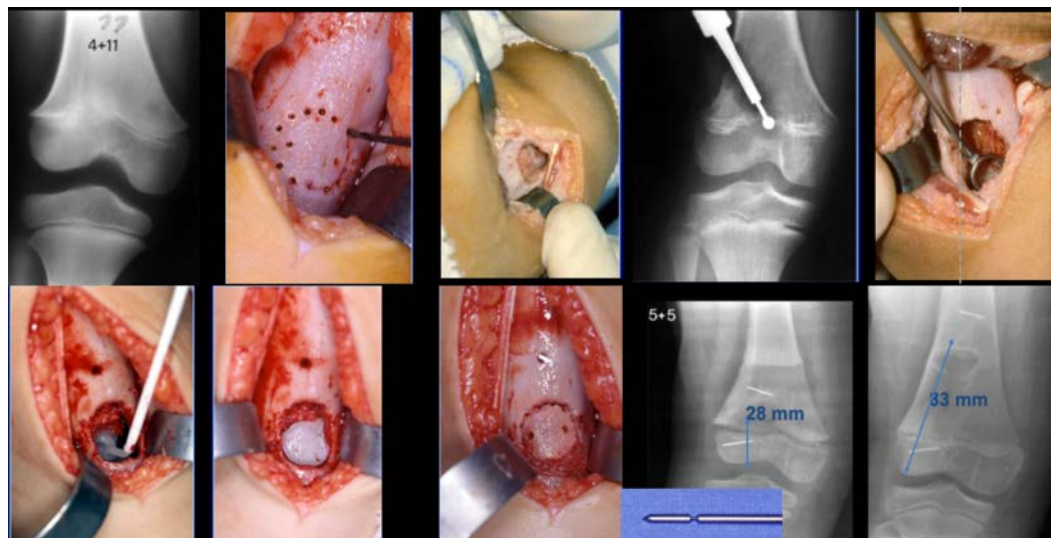


Figure 14. Physal Bar Resection of a central bar involving the distal femoral physis. A large metaphyseal cortical window is created to allow removal with a burr (top row). Cranioplast is placed across the physal defect and the cortical window replaced. Pins left at the time of surgery can aid in postoperative radiographic assessments.

Bioengineering and the Physis – The Role of Stem Cells and Scaffolds

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While injuries around the growth plate may often heal, occasionally the growth potential of the physis is lost directly by the injury or as a consequence to the healing process. Replacing the injured portions of the growth plate remains a hurdle that has not yet been solved. Tissue engineering strategies can be employed to attempt to accomplish this goal. In general terms, tissue engineering involves stem cells, scaffolds, and signals.

Several different types of stem cells can be used in such applications such as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult somatic stem cells, of which mesenchymal stem cells (MSCs) are included. MSCs may be derived from multiple tissues, including, but not limited to, umbilical cord blood, adipose tissue, and bone marrow. Among them, bone marrow-derived MSCs are commonly used to promote healing for musculoskeletal regeneration. The MSCs can do this by (1) contributing to tissue formation, i.e., becoming part of the repaired tissue through cell differentiation, or (2) modulating the microenvironment through mediating the production of trophic factors.

Scaffolds used to load stem cells and keep them at a desired site of implantation are critical in tissue

engineering. They serve the purpose of filling tissue defects, offering structural support, and conveying structural signals. In terms of chondrogenesis, an ideal scaffold must promote the phenotypic shape of chondrocytes and accommodate a high cell density. To date, many scaffolds made of different materials have been used in attempts of tissue-engineering growth plate, the most common being various forms of hydrogels.

The two biggest challenges that need to be overcome in developing a functional tissue-engineered growth plate include (1) incomplete differentiation of stem cells and (2) partial morphogenesis of the growth plate. Incomplete differentiation occurs when stem cells fail to fully differentiate into terminal chondrocytes and partial morphogenesis occurs when the typical zonal distribution found in the physis fails to occur. From a given pool of stem cells, a heterogeneous population of cell derivatives develop as stem cells differentiate at different rates. This problem may be mitigated by choosing appropriate stem cell types (i.e., bone marrow-derived MSCs more effectively progress to hypertrophic chondrocytes than pluripotent stem cells) and by fully understanding proper sequential and temporal signals, such as growth factors and mechanical loading, required for induction of differentiation at different stages of growth plate development.

Current Surgical Treatments to Address Physeal Abnormalities in Skeletal Dysplasias

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Traditional treatment methods for lower limb deformity in children with limited growth or “pathologic physes” calls for osteotomies to correct these deformities. The inference that the physis is “sick” implied a contraindication for surgical manipulation of the physis. We think of deformities in different parts of the bone and what conditions typically present with different dysplasias. For instance, coxa vara is noted in spondyloepiphyseal dysplasia congenita (SEDC), Kniest dysplasia, and Spondylometaphyseal dysplasia (SMD) (Figure 15). On the other hand, coxa valga is seen in Morquio syndrome. At the knee, genu varum is seen in Achondroplasia, Multiple Epiphyseal Dysplasia (MED), and Pseudoachondroplasia. Genu valgum is seen in Morquio syndrome, MED, pseudoachondroplasia, and Multiple Hereditary Exostosis (MHE) (Table 1).

Coxa Vara	Coxa Valga
Spondyloepiphyseal Dysplasia Congenita (SEDC) Kniest Dysplasia Spondylometaphyseal (SMD)	Morquio
Genu Varum	Genu Valgum
Achondroplasia Multiple Epiphyseal Dysplasia Pseudoachondroplasia	Morquio Syndrome Multiple Epiphyseal Dysplasia Pseudoachondroplasia Multiple Hereditary Exostosis (MHE)

Table 1. Typical deformities treated in different skeletal dysplasias

When treating these patients, we aim for aligning the mechanical axis and need to consider the methods of correction, which includes consideration of both guided growth and osteotomies. Early intervention with guided growth in skeletal dysplasia patients should be done earlier as they correct slower than patients of average stature. As a general guideline, mild to moderate limb

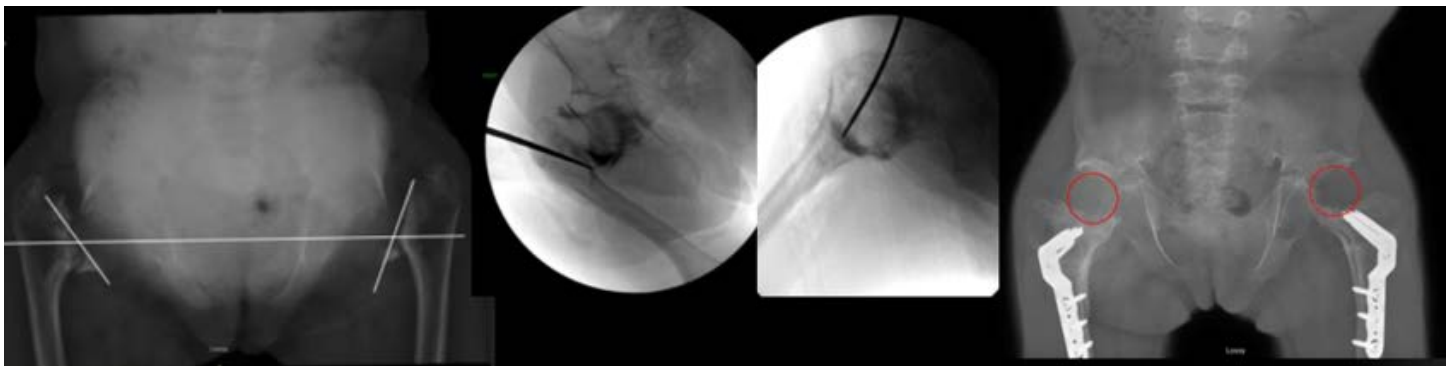


Figure 15. Coxa vara exists in numerous pediatric conditions, including skeletal dysplasias. The delayed ossification of the femoral head can make operative treatment challenging. An intraoperative arthrogram can be used to greatly assist the correct placement of implants, as was performed in this 11 year old with SMD.

deformity can be corrected in skeletal dysplasia patients using guided growth between 3-6 years of age. Older patients have less growth remaining and guided growth will have little to no impact on correction of the deformity (Figure 16). Skeletal dysplasia patients with substantial angular deformity or those older than 7 years of age might have a more predictable result after an osteotomy. Corrective osteotomies can be performed and acutely stabilized with internal fixation or with an external fixator in the older skeletal dysplasia population.

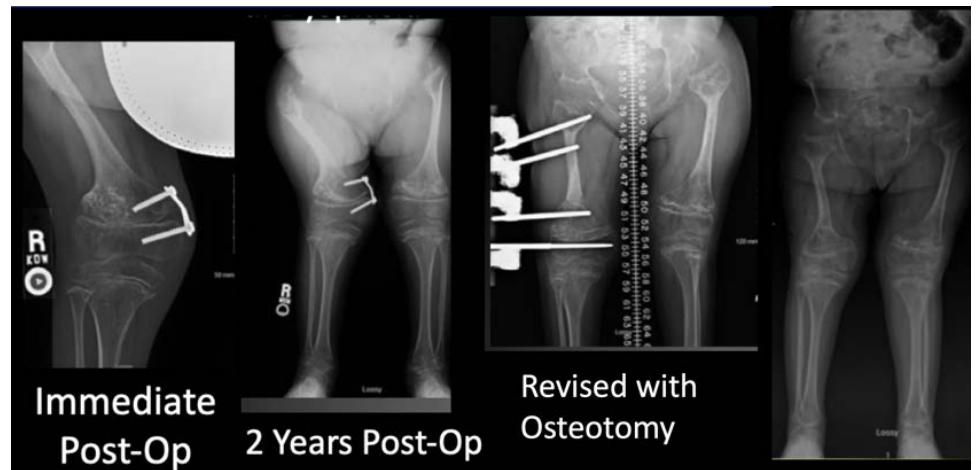


Figure 16. Deformity correction via osteotomy is preferred when angular deformities exist in the presence of a physeal bar or in patients that lack enough growth to respond to “guided growth” techniques. Osteotomies provide effective angular correction as demonstrated in this case of a valgus deformity in a 12 year old with Spondyloepiphyseal dysplasia that failed initial guided growth treatment.

Surgical Treatment of Angular Deformities: Guided Growth

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Without constraints, skeletal growth would be centrifugal, as seen in the carpal and tarsal bones that (except the calcaneus) do not have a physis. At each end of the long bones, the physis is constrained at the perimeter by the perichondrial ring of LaCroix (Figure 17). This accommodates longitudinal growth, which ultimately determines stature and symmetry. With the exception of the proximal femur, the lower extremity physes are oriented parallel to the ground, thus perpendicular to gravity, in the standing position. The cartilaginous, columnar physis is mechanically well suited to accommodate intermittent and physiologic compressive loads but less resilient to excessive torsional or shear forces.

Historically, a corrective osteotomy has been the treatment of choice for most angular limb deformities. A wide variety of implants and external fixators have been

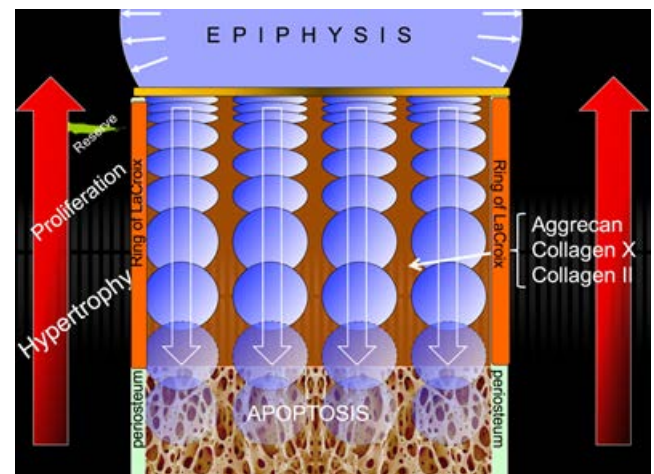


Figure 17. The epiphysis tends to grow circumferentially, not unlike the tarsal bones. The ring of LaCroix constrains the perimeter of the physis, resulting in longitudinal growth of the metaphyses. The vertical columns of cartilaginous cells, supported within a normal collagen matrix, are best suited to resist vertical, intermittent, physiologic loads.

developed to stabilize the bone segments and maintain alignment during healing. However, this invasive modality is expensive, associated with complications, and ignores the growth potential of the physis. Unfortunately, recurrent deformity is not rare, and osteotomies are not always definitive.

The concept of harnessing the growth potential of the physis to correct deformities was first described by Phemister in 1933. While no implant was employed, the permanent ablation of the physis limited its practical use to adolescent patients. The staples, introduced by Blount, enjoyed several decades of popularity. However, by placing a rigid implant around such a dynamic structure, implant migration, breakage, and bending often occurred. It is also unappealing to violate the physis with large diameter threaded screws (PETS). The limitations imposed by rigid implants that restrict or compress the physis include unplanned revision surgery and sometimes difficult implant retrieval. This led to the idea of using a flexible and dynamic implant, consisting of a plate and screws. Over the past decade, redirecting any open physis by means of extraphyseal flexible tethering has obviated the need for osteotomies in most growing children. Applications extend to any age, any direction, any long bone(s), and any etiology. This technique has been successful in children ages 18

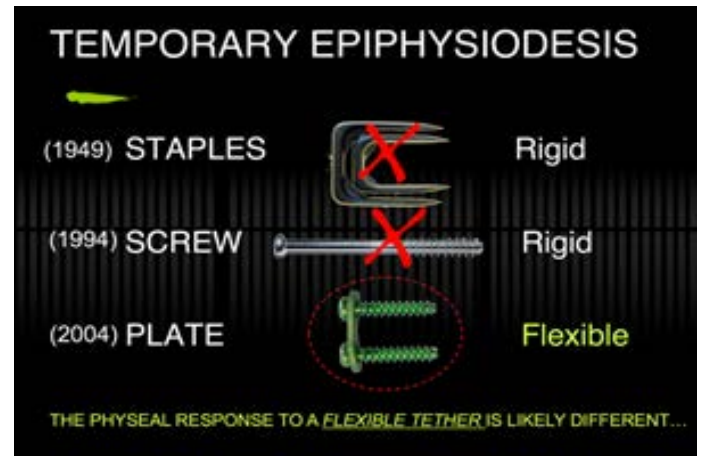


Figure 18. Guided growth has evolved from the use of rigid implants that compress the physis in favor of a peripheral tension band to tether it. This has extended the applications and broadened the age range.

months to 18 years, given that the physis is still growing (Figure 18).

By consensus, surgical intervention is inappropriate for physiologic deformities (i.e., genu varum < 2 years and genu valgum < 6 years). In contradistinction, pathologic deformities are apt to progress, causing pain and compromising gait. Depending upon the etiology and progression of the angular deformity, guided growth may be initiated, even in very young children. When the mechanical axis has been restored to neutral, providing

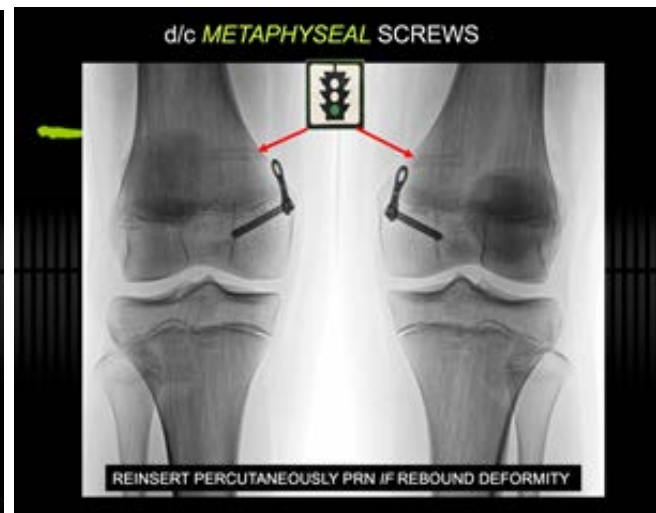
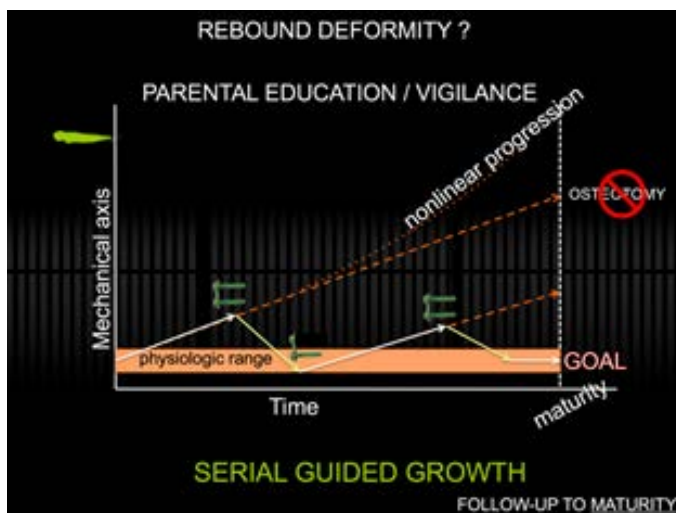


Figure 19. Following implant removal (metaphyseal screw vs. plate), rebound deformity

the plate remains in good position and the epiphyseal screw is not infringing upon the physis, the metaphyseal screw may be removed percutaneously. Should rebound deformity become evident, the screw can be reinserted. This concept of intermittent guided growth is simple, minimally invasive, and economical (Figure 19).

The worldwide success of guided growth has averted the cost and complications of routinely performing osteotomies for the correction of pediatric limb deformities. Simultaneous bilateral and multi-level

correction is feasible, typically on an outpatient basis. No immobilization is imposed because no bone healing is required. Suffice it to say that the biology of temporarily restraining one side of the physis with an extra periosteal tension band is more appealing than applying a rigid restraint. However, whether the deformity is idiopathic, metabolic, congenital, or post-traumatic in nature, we need better elucidation of what is going on within the physis, before, during, and following treatment (in some cases). This lends appeal to the concept of serial physal biopsy.

Evening out Limbs: Epiphysiodesis and Distraction Osteogenesis

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When treating leg length discrepancies there are two main options: make the short leg longer through distraction osteogenesis or shorten the long leg with acute skeletal shortening (in adults) or via an appropriately timed epiphysiodesis (in children). Epiphysiodesis can be utilized for projected discrepancies of 2.5-5cm, which has been done since the early 1900s. Percutaneous epiphysiodesis has been described and works well. Percutaneous screws have been tried but seem to result in a slower time to retard growth as well as other problems, including prominent hardware and iatrogenic deformity. Temporary epiphysiodesis via tethering with staples or plates has also been tried and to date has not been as reliably successful. Animal models have tried using radiofrequency ablation on the physis. In the pig model, there was minimal pain and complete closure of the physis at six months. These techniques have yet to be advanced to humans.

Limb lengthening can be accomplished with distraction osteogenesis (Figure 20). Indications include final leg length discrepancies of 5-15cm, a final discrepancy of

>3 cm with concomitant deformity that requires an osteotomy to correct the deformity or bone loss secondary to trauma, infection, or tumor. Classically, this has been accomplished with external fixation and lengthening with the frame. Limb lengthening is a labor-intensive process for the patient, family, and surgeon; and results in multiple complications. Limb lengthening obstacles are common and include problems with muscle growth and resultant muscle damage and tightness. Lengthening can result in muscle pain, weakness, decreased joint motion resulting in dislocated joints, and articular cartilage damage. Prevention of muscle complications can be done by performing soft tissue releases, expanding the external fixator to the other side of the knee or ankle joint.

Recent advances in limb lengthening include the use of intramedullary nails which can be remotely lengthened via the use of magnetic forces. These devices have revolutionized limb lengthening and have decreased rates of complications as seen with external fixation and the transfixing pins and wires that are used. In 2016, Laubscher et al. compared the results of femoral

lengthening with the Precice nail to standard external fixation. Greater patient satisfaction and lower rates of complications were noted with intramedullary lengthening.²⁴ However, the “Holy Grail” of pediatric orthopaedics still eludes us. What are the biological mechanisms whereby limbs grow larger in hemihypertrophy or longer after peri-epiphyseal trauma, tumors, or infection? Once identified and harnessed in many children, we will no longer be required to shorten the good leg or perform invasive procedures on the shortened limb.



Figure 20: A 9-year-old girl with a congenital short femur has undergone lengthening with a Precice nail (NuVasive, San Diego, CA).

Conclusions

When the conference was over, it was clear that the conversation regarding our understanding of the physis had only started. The co-directors gave the attendees homework to identify the largest gaps of understanding so that these could be compiled and shared moving forward. Participants felt that a formal growth plate study group within the ORS should be explored, or that a biennial physeal conference should be created to allow further collaborations and to present research progress.

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References

- Eastwood DM, Sanghrajka AP. Guided growth: recent advances in a deep-rooted concept. *J Bone Joint Surg Br* 2011;93(1):12-8 doi: 10.1302/0301-620X.93B1.25181[published Online First: Epub Date]].
- Lin Z, Rodriguez NE, Zhao J, et al. Selective enrichment of microRNAs in extracellular matrix vesicles produced by growth plate chondrocytes. *Bone* 2016;88:47-55 doi: 10.1016/j.bone.2016.03.018[published Online First: Epub Date]].
- Nowlan NC, Murphy P, Prendergast PJ. A dynamic pattern of mechanical stimulation promotes ossification in avian embryonic long bones. *J Biomech* 2008;41(2):249-58 doi: 10.1016/j.jbiomech.2007.09.031[published Online First: Epub Date]].
- Ratliff AH. The short leg in poliomyelitis. *J Bone Joint Surg Br* 1959;41-B(1):56-69
- Walsh M, Connolly P, Jenkinson A, O'Brien T. Leg length discrepancy--an experimental study of compensatory changes in three dimensions using gait analysis. *Gait Posture* 2000;12(2):156-61 doi: 10.1016/S0966-6362(00)00067-9[published Online First: Epub Date]].
- Killion CH, Mitchell EH, Duke CG, Serra R. Mechanical loading regulates organization of the actin cytoskeleton and column formation in postnatal growth plate. *Mol Biol Cell* 2017;28(14):1862-70 doi: 10.1091/mbc.E17-02-0084[published Online First: Epub Date]].
- Aszodi A, Hunziker EB, Brakebusch C, Fassler R. Beta1 integrins regulate chondrocyte rotation, G1 progression, and cytokinesis. *Genes Dev* 2003;17(19):2465-79 doi: 10.1101/gad.277003[published Online First: Epub Date]].
- Terpstra L, Prud'homme J, Arabian A, et al. Reduced chondrocyte proliferation and chondrodysplasia in mice lacking the integrin-linked kinase in chondrocytes. *J Cell Biol* 2003;162(1):139-48 doi: 10.1083/jcb.200302066[published Online First: Epub Date]].
- Song B, Haycraft CJ, Seo HS, Yoder BK, Serra R. Development of the post-natal growth plate requires intraflagellar transport proteins. *Dev Biol* 2007;305(1):202-16 doi: 10.1016/j.ydbio.2007.02.003[published Online First: Epub Date]].
- Abad V, Meyers JL, Weise M, et al. The role of the resting zone in growth plate chondrogenesis. *Endocrinology* 2002;143(5):1851-7 doi: 10.1210/endo.143.5.8776[published Online First: Epub Date]].
- Nilsson O, Weise M, Landman EB, Meyers JL, Barnes KM, Baron J. Evidence that estrogen hastens

epiphyseal fusion and cessation of longitudinal bone growth by irreversibly depleting the number of resting zone progenitor cells in female rabbits. *Endocrinology* 2014;**155**(8):2892-9 doi: 10.1210/en.2013-2175[published Online First: Epub Date]].

12. Marino R, Hegde A, Barnes KM, et al. Catch-up growth after hypothyroidism is caused by delayed growth plate senescence. *Endocrinology* 2008;149(4):1820-8 doi: 10.1210/en.2007-0993[published Online First: Epub Date]].

13. Bonafe L, Cormier-Daire V, Hall C, et al. Nosology and classification of genetic skeletal disorders: 2015 revision. *Am J Med Genet A* 2015;167A(12):2869-92 doi: 10.1002/ajmg.a.37365[published Online First: Epub Date]].

14. Mortier GR, Cohn DH, Cormier-Daire V, et al. Nosology and classification of genetic skeletal disorders: 2019 revision. *Am J Med Genet A* 2019;179(12):2393-419 doi: 10.1002/ajmg.a.61366[published Online First: Epub Date]].

15. Pauli RM. Achondroplasia: a comprehensive clinical review. *Orphanet J Rare Dis* 2019;14(1):1 doi: 10.1186/s13023-018-0972-6[published Online First: Epub Date]].

16. Ornitz DM, Legeai-Mallet L. Achondroplasia: Development, pathogenesis, and therapy. *Dev Dyn* 2017;246(4):291-309 doi: 10.1002/dvdy.24479[published Online First: Epub Date]].

17. Komla-Ebri D, Dambroise E, Kramer I, et al. Tyrosine kinase inhibitor NVP-BGJ398 functionally improves FGFR3-related dwarfism in mouse model. *J Clin Invest* 2016;126(5):1871-84 doi: 10.1172/JCI83926[published Online First: Epub Date]].

18. Peck SH, O'Donnell PJ, Kang JL, et al. Delayed hypertrophic differentiation of epiphyseal chondrocytes contributes to failed secondary ossification in

mucopolysaccharidosis VII dogs. *Mol Genet Metab* 2015;**116**(3):195-203 doi: 10.1016/j.ymgme.2015.09.008[published Online First: Epub Date]].

19. Schuchman EH, Ge Y, Lai A, et al. Pentosan polysulfate: a novel therapy for the mucopolysaccharidoses. *PLoS One* 2013;8(1):e54459 doi: 10.1371/journal.pone.0054459[published Online First: Epub Date]].

20. Smith LJ, Martin JT, O'Donnell P, et al. Effect of neonatal gene therapy on lumbar spine disease in mucopolysaccharidosis VII dogs. *Mol Genet Metab* 2012;107(1-2):145-52 doi: 10.1016/j.ymgme.2012.03.013[published Online First: Epub Date]].

21. Wöhrle S, Henninger C, Bonny O, et al. Pharmacological inhibition of fibroblast growth factor (FGF) receptor signaling ameliorates FGF23-mediated hypophosphatemic rickets. *J Bone Miner Res* 2013;28(4):899-911 doi: 10.1002/jbmr.1810[published Online First: Epub Date]].

22. Wang DC, Deeney V, Roach JW, Shah AJ. Imaging of physeal bars in children. *Pediatr Radiol* 2015;45(9):1403-12 doi: 10.1007/s00247-015-3280-5[published Online First: Epub Date]].

23. Yuan BJ, Stans AA, Larson DR, Peterson HA. Excision of Physeal Bars of the Distal Femur, Proximal and Distal Tibia Followed to Maturity. *J Pediatr Orthop* 2019;39(6):e422-e29 doi: 10.1097/BPO.0000000000001349[published Online First: Epub Date]].

24. Laubscher M, Mitchell C, Timms A, Goodier D, Calder P. Outcomes following femoral lengthening: An initial comparison of the Precice intramedullary lengthening nail and the LRS external fixator monorail system. *Bone Joint J* 2016;98-B(10):1382-88 doi: 10.1302/0301-620X.98B10.36643[published Online First: Epub Date]].